MYCOLOGIA

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MYCOLOGY AND MEDICINE[1]

CHESTER W. EMMONS

One year ago, as your retiring president, I had the privilege of addressing the Mycological Society upon an aspect of mycology which has occupied my attention since 1929. It was my thesis then that medical mycology is not an abstruse specialty of medical microbiology and clinical medicine. It is an integral part of mycology. It embraces the study of some of the most familiar and most nearly ubiquitous molds as well as the few rare fungi which are known almost exclusively from human and animal hosts. It is the responsibility of mycologisis to supply active and informed leadership in this branch of mycology (1).

Today, faced by the responsibility of presenting an address worthy of the traditions of the Annual Lecture, I was tempted to repeat for emphasis some aspects of the challenge I offered a year ago. Repetition, however, is tedious and, in any case, I propose to discuss instead some more remote associations between mycology and medicine than those presented by the mycoses alone. The historical aspects of these relationships reflect man's prescientific groping toward an understanding of the causes of disease and a search for defenses against illnesses. Current ramifications include brilliant but partial successes in achieving these goals.

In the credulous practices of medical folklore, in the traditional and recorded precepts of Hippocrates, Dioscorides, Sartorius and other practitioners of the healing arts 2000 years ago, in the herbals and among the medicinal drugs of the herbalist, in the pharmacopoeias of more re-

[1] Presented as the Twelfth Annual Lecture of the Mycological Society of America, West Lafayette, Indiana, August 30, 1961.

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cent time, and in the modern medical era of antibiotic chemotherapy, the fungi have held secure positions.

In his Presidential Address before the British Mycological Society in 1914, Prof. A. H. R. Buller presented a scholarly and erudite review of the medicinal, culinary and criminal uses of fungi by the Greeks and Romans (**2**). From the quotations which he gleaned during many years of bibliographic research, from Rolfe and Rolfe's "The Romance of the Fungus World" (**3**), from Badham's "A Treatise on the Esculent Funguses of England" (**4**), I have drawn freely. From these sources I can review here only a few of the medicinal uses to which fungi were put by man during the prescientific eras of medical practice. The courtesy and assistance of Mr. John A Stevenson and access to his extensive mycological library have been indispensable aids in the preparation of this lecture.

The magical uses of fungi may be exemplified by the superstitious practice of carrying *Daldinia concentrica* in the pocket as protection against cramps. The rationale of the imagined efficacy of the mandrake has a counterpart in the ancient use of *Auricularia auricula* for inflammation and diseases of the throat. Therapeutic activity was hopefully assumed because of the fancied resemblance of the hymenial surface of the fungus to the anatomical structures of the upper throat. Its subjective effect, if not its efficacy, may have been enhanced by its ingestion after being "boyled in milk, steeped in beere, vinegar, or any other convenient liquor." *Elaphomyces granulatus, Phallus impudicus* and other fungi were prized as essential components of love potions.

Some fungi received more official recognition from the physician. Dioscorides, in De Medicina, extolls *Polyporus officinalis (Fomes officinalis)* as a panacea. In the translation quoted by Buller he states: "Its properties are styptic and heat-producing, efficacious against colic and sores, fractured limbs, and bruises from falls: the dose is two obols weight with wine and honey to those who have no fever; in fever cases with honeyed water; it is given in liver complaints, asthma, jaundice, dysentery, kidney diseases where there is difficulty in passing water, in cases of hysteria, and to those of a sallow complexion, in doses of one drachma; in cases of phthisis it is administered in raisin wine, in affections of the spleen with honey and vinegar." He recommended it too for pains in the stomach and acid eructations, to stop bleeding, for pains in the joints, as a purgative, as an antidote for poisons, for snake-bite, and he summarized its virtues by stating: "On the whole it is serviceable in all internal complaints when taken according to the age and strength of the patient; some should take it with water, others with wine, and others with vinegar and honey, or with water and honey."

According to Pliny, *Boletus edulis* also was useful in a wide variety of illnesses, being recommended for diarrhea, condylomata, freckles, "blemishes on women's faces," sore eyes, ulcers and dog bites.

Within the past 100 years Western physicians were still using *Fomes officinalis, Trametes suaveolens, Lactarius piperatus* and *L. torminosus* in the treatment of tuberculosis. In fact "The Dispensatory of the United States of America, 1950" still listed Agaric, defined it as, "Agaric is the dried fruit body of *Polyporus officinalis* Fries, deprived of its outer rind," stated that it was collected in larch forests of Russia and exported to Hamburg and continued, "The most important use of agaric has been in the treatment of the colliquative sweats of wasting conditions such as phthisis." The quotation concludes with the following delightful example of cautious conservatism: "Opinion has differed as to its value" (**5**). The Dispensatory devoted another paragraph to the "surgeon's agaric," *Polyporus fomentarius (Fomes fomentarius)*, which was used to arrest hemorrhage. The National Formulary listed agaric in the 1916 edition (**6**) and agaric acid was listed in New and Non-official Remedies in 1921 (**7**). The Formulary defined agaric acid as a tribasic acid ($C_{19}H_{36}OH$-$(COOH)_3 \cdot 1\frac{1}{2}H_2O$) derived from *Polyporus officinalis* which, "paralyzes the peripheral nerves of the sweat glands, arresting the secretion of sweat" and stated that it has only 1/20 the activity of atropine.

Perhaps it ill behooves us to disparage the use of these fungi in the treatment of tuberculosis when we remember that it is only within the recent past that safe and effective chemotherapy against tuberculosis has become available through the research and rigid testing methods of the laboratory, experimental use of animals and clinical trials with "double blind" controls.

The "agarick" or "agaricum" of the ancients was a "universal drug". Although it rose as a relic of the past in twentieth century medicine, the spectrum of its alleged efficacy was greatly diminished. In one of its principal uses as a cathartic, it undoubtedly had a certain but unpredictable degree of efficacy. Many other fungi, as any mycophagist knows, are emetics, but fortunately they are replaced in modern medicine by drugs of known potency. In most of their other medicinal uses the natural remedies of the herbalist had less obvious effects, their use was based upon tradition, and the dependence of the physician upon them did not have the support of even empirical success.

Only two or three generations intervene between modern medicine and the practice of bleeding by cupping or use of leeches. In that same era cauterization also was practiced and, indeed, the physician today finds the use of counterirritants advisable under some circumstances and the

practice is common in self medication by the lay public. Fungi were widely used since the days of Hippocrates as severe counterirritants in producing blisters. In this capacity *Fomes officinalis,* as well as *Fomes fomentarius,* species of *Lycoperdon, Calvatia* and other puffballs were used. Again I quote Buller in a translation of Hippocrates, "One should cauterize the osseous and nervous parts with fungi"; "Quickly cauterize in eight places so as to intercept the extremities of the spleen" and "When the liver has attained its greatest volume, one should cauterize with fungi."

This use of fungi depended upon the slow burning quality of dry fungi. In later times this quality was increased or partially standardized by slicing, drying, pulverizing, soaking in saltpetre and again drying suitable fungi. The production of tinder by this method was at one time an important industry. The fungal tinder so prepared was placed upon the skin, ignited and (if the patient's stoicism permitted) was allowed to burn until a large blister was produced into which, hopefully, the toxins and the pain generated by the disease were drained from the body.

This method of cauterization apparently persisted amongst primitive practitioners after the more "enlightened" practice of bloodletting by leeches was accepted by English physicians. J. D. Hooker in his Himalayan Journals, as quoted by Rolfe, relates that in Sikkim in 1850: "My servant having severely sprained his wrist by a fall, the Lepchas wanted to apply a moxa, which they do by lighting a piece of puffball, or Nepal paper that burns like tinder, laying it on the skin and blowing it till a large open sore is produced; they shook their heads at my treatment, which consisted in transferring some of the leeches from our persons to the inflamed part."

The spores and capillitium of puffballs were used in ancient time for their styptic properties and to hasten production and enhance adherence of eschars. It is probable that the application of dried puffballs to a bleeding wound is still an important item in the "rough and ready" first aid kit of rural home treatment in many parts of the world. Rolfe stated in 1925 that puffballs had been used until a few years before as haemostats and surgical dressings and that they were still used by dentists as absorbents in preparing dental cavities for filling. The more recent scientific testing of the oncolytic or tumor dissolving properties of *Calvatia* spp. is known to many of you.

Fascinating as the subject is I can not at this time delve further into the folklore and early medicinal uses of fungi. In the art and science of medical practice today, traditional use of natural plant products and of

drugs has been largely but not entirely replaced by dependence upon drugs of known purity and potency which have been tested in controlled clinical trials. If adherence to this ideal is not universal, the failure is due to laboratory tests which are sometimes equivocal and disputed, to the physician's inability to separate true and false in the mass of promotional literature which floods his office and to the public's demands for "miracle drugs" which may indeed be highly efficacious in a specific disease but are not necessarily suitable to the condition for which the patient seeks relief.

Four fungi, *Claviceps purpurea, Ustilago maydis, Polyporus officinalis* and *Saccharomyces cerevisiae*, retained places in formularies or pharmacopoeias into the twentieth century as crude natural products. The dried fungi are now replaced by purified extracts, derivatives or synthetic analogs, except for yeast which has wide use as a food supplement. Yeast at one time was used in poultices. Its actual medicinal values probably reside in its nutrient and vitamin content and its use in self medication as a food supplement probably far exceeds its use in prescriptions.

Claviceps purpurea, by transformation of the ovary and embryo of rye into a fungal sclerotium, produces the ergot of commerce. Other species of *Claviceps*, on other hosts, also produce ergot, usually of lower potency. The toxic results of eating bread made from rye flour contaminated with ergot have been recognized for several hundred years and the peasant, forced in times of famine to eat bread made from such flour, was afflicted with gangrenous skin lesions, central nervous system disease and other symptoms of toxemia. The pharmacological effects of ergot taken in therapeutic doses include increased blood pressure, but the most important medicinal use is in obstetrics to increase the intensity and duration of uterine contractions. The dosage of crude ergot was uncertain because of varying amounts and proportions of several alkaloids and decay of the active principles upon storage. Thanks to the chemist, the physician can now select and prescribe the particular alkaloid whose activity is desired.

The Pharmacopoeia of the United States, 6th Edition, 1882, listed *Ustilago* without comment related to its pharmacologic properties. The Dispensatory of the United States, 24th Edition, 1947, in contrasting ergot and ustilago stated "while they both have the power of causing contractions of the uterine muscle, their other actions are quite different and there is no reason to suspect that any of the ergot principles occur in this drug." The Dispensatory briefly reviewed diverse opinions regarding the oxytocic and other alleged pharmacologic activities of ustilago.

It seems to be the consensus among veterinarians that the fungus is not toxic for cattle and some mycophagists are accustomed to its use as food.

The ingestion of toxic, sublethal and lethal meals of mushrooms supplies another link between mycology and medicine. Both Hippocrates and Dioscorides record cases of mushroom poisoning and Greek and Roman mythology and history contain many references to accidental or intentional use of toxic fungi. According to tradition the Berserkers induced frenzies by eating *Amanita muscaria* and similar uses of this and other fungi are reported for some Siberian tribes within historical times.

Recognition that some fungi were edible delicacies while others were deadly poisons lead the ancient philosopher to attempt an explanation of this difference. Dioscorides stated that the toxic fungi, "grow either amongst rusty nails or rotten rags or near serpents holes, or on trees producing noxious fruits." Pliny repeated this opinion and, referring to toxic mushrooms stated, "if, for instance, the hole of a venomous serpent be near, and the serpent breathe upon them as they open, from their natural affinity with poisonous substances they are readily disposed to imbibe such poison. Therefore it will be well to exercise care in gathering them until the serpents retire into their holes."

Among more lethal mushrooms gastrotoxic, neurotoxic and hemolytic toxins are recognized. Ford described five types of poisoning based upon the predominance of one or upon varying combinations of more than one toxin in the mushrooms ingested (**8**). According to Ford's classification *Mycetismus gastro-intestinalis* follows ingestion of *Russula emetica, Boletus satanus, B. miniato-olivaceus, Lactarius torminosus, Entoloma lividum* and *Lepiota morgani,* and is characterized by nausea, vomiting and diarrhea. It usually is followed by recovery even without therapy in 1–2 days.

Mycetismus choleriformis follows ingestion of *Amanita phalloides* and is the cause of 90 per cent of the 50 or more deaths reported annually on the average in the United States. *Pholiota autumnalis, Hygrophorus conicus* and a few other species of *Amanita* contain the same toxin which is thermostable and has a phosphorus-like action. A delay of 6–15 hours in the appearance of symptoms is partly responsible for the high fatality rate of 50 per cent.

Mycetismus nervosus follows ingestion of *Amanita muscaria, A. pantherina, Inocybe infelix, I. infida, Clitocybe illudens* and others. The principal toxin, muscarin, is a heat stable substituted ammonia base similar to choline and it stimulates the parasympathetic system in a manner similar to that of nicotine and curare. In appropriately small doses mus-

carin has an intoxicating effect similar to that of marihuana obtained from *Cannabis sativa*.

Mycetismus sanguinareus is caused by glucosidal hemolysins which are heat resistant and may occur after eating *Helvella esculenta* and several other fungi unless they are well cooked. *Mycetismus cerebralis* has been discussed under the subject of hallucinogenic mushrooms.

Hemorrhagic diseases caused in horses and cattle by ingestion of moldy hay and fodder are well known to veterinarians and have been most extensively studied by Russian scientists (**9**). Man may become ill after contact with or inhalation of the molds, or after ingestion of moldy cereal grains. *Stachybotris alternans, Fusarium roseum* and *F. sporotrichioides* are believed to be the principal agents of this type of poisoning.

In last year's Annual Lecture Mr. R. Gordon Wasson presented dramatically a scholarly review of the historical and current uses in religious and social rites of the hallucinogenic mushrooms (**10**). He reported his personal investigations of the ritual use of the "sacred mushroom" by Indians in Mexico, Guatemala and other Latin American countries in traditional ceremonies older than history. The curanderas or priestesses, with varying degrees of skill and reverence, partake of the "sacred mushroom" and then profess to gain extrasensory perception. Even the novice who participates in the meal has remarkable visual, auditory and mental hallucinations. The mushrooms used vary with the area and include species of *Conocybe, Panaeolus, Psathyrella, Psilocybe* and *Stropharia*. Some of the active alkaloids in these mushrooms appear to be the same as those present in certain seeds which have long been used in Mexico to induce trances. Fractions, derivatives and analogs of the alkaloids found in these mushrooms are now under study for possible use in psychiatric medicine.

Probably few people in the United States realize the extent to which mushrooms have been used as staple foods in other parts of the world. This is somewhat outside the scope of my title, except that mycologists who have been asked to identify mushrooms incriminated in a toxic meal are well aware that one of the most frequent circumstances involved is the inclusion in his meal of a species resembling an innocuous mushroom which the gatherer was accustomed to collecting and eating in his native European country. Duggar reported that in 1901 Munich was the principle mushroom market in Europe, that 31 species of mushrooms were sold there commercially and that the total marketed crop for that year was nearly 1000 tons (**11**). This extensive use of mushrooms as food and the occasional inclusion of toxic species led to legal regulations of the

market and reduction of permitted species. In some countries custom or legislation limited sales to only a few species and it is reported that Mohammedans ate only morels, rejecting other mushrooms as unfit for food.

If in this portion of my allotted time I have dwelt too long on the past, I can only hope that you share with me an interest in the mores and habits of ancient man and the emergence of objective testing methods from the irrational practices of traditional medicine. Fungi were a part of the traditional and ineffective armamentarium of the herbalist, but during the past 15 years the fungi have supported a revolution in the therapy of infectious diseases.

Based upon Fleming's observation of the antibiotic action of *Penicillium notatum* in a contaminated culture plate and the investigations and discoveries of his associates and of countless other scientists, the molds and the actinomycetes have yielded a host of drugs which are more effective against the bacterial invaders of man's body than those which come from the chemist's laboratory. In 1959, 2,295,000 pounds of antibiotics were produced for human and animal use at a market value of $318,188,000 (**12**). A conservative estimate of the cost of research and development (supported by universities, pharmaceutical houses and government subsidies) extending over the past 20 years must be measured in billions of dollars.

Many of the antibiotics which are active *in vitro* are too toxic for therapeutic use, some which are effective in experimentally infected animals and in treatment of human disease are selective in their action, others exhibit broad spectrums of activity. Three antibiotics, mycostatin, amphotericin-B and an as yet unnamed antibiotic (X-5079C) are partially effective against mycoses (**13**, **14**, **15**). Better antibiotics are still being sought. Man's conquest against disease is not static. After the first dramatic success of penicillin, so-called "hospital" or penicillin resistant strains of *Staphylococcus* appeared. The chemist currently has the advantage by his creation of derivatives of penicillin which are effective against the resistant strains, but the bacteria will almost certainly score again.

Therapeutic use of the antibiotics has almost eliminated as causes of death in the United States many of the deadly infectious diseases of a few years ago. Typhoid, which killed 2659 persons in the United States in 1937 was reported as a cause of death in only 34 cases in 1957 (**16**). These therapeutic triumphs have not been achieved in the mycoses. Indeed the reported deaths from mycoses have held between 350 and 450 since they have been effectively reported. Amphotericin-B and X-5079C can be credited with a number of cures, but to the present these are ap-

proximately balanced by more frequent diagnoses of fungus infections and perhaps by an actual increase in numbers of fatal mycoses. As the life span of man is increased by the conquest of infectious disease and even of cancer, fungi (for which fully adequate control is still lacking) may parasitize the older or debilitated patient. Leukemia, diabetes and other malignant diseases decrease the defense of the patient and even some types of desirable therapy, unless judiciously used, increase the susceptibility of man to fungus infections. Conquest of the mycoses remains a serious medical problem.

So we arrive in this review at the most direct and obvious association between mycology and medicine, the invasion by fungi of the body cavities and organs of man. Fungi were among the earliest recognized agents of disease. Shortly after Bassi observed fungi in muscardine of silkworms in 1835, the fungi of thrush, of favus and of several types of ringworm were described. The fascinating but oft reviewed history of medical mycology need not be again related here. Today we admit to the list of well recognized fungal pathogens of man more than 50 species of fungi and we recognize that additional species, under special conditions of implantation or in an abnormally susceptible patient may cause disease. In most of the mycoses a clinical diagnosis is provisional and needs confirmation in the laboratory. The numbers of trained mycologists in hospital laboratories are still very inadequate.

The mycologist in a diagnostic laboratory should have training and experience in general mycology, although the excellent intensive short courses now available enable the interested technician without more extensive experience to do a creditable job. Diagnostic mycology, however, still is a developing science and requires research ability and orientation if the opportunities for scientific studies of the mycoses are to be fully exploited. Research studies related to case studies of mycoses, to focal outbreaks of multiple cases and to epidemiologic patterns of mycoses have elucidated many of the features which distinguish the mycoses from many infectious diseases.

The systemic mycoses are not contagious and the etiologic agents grow and persist as saprophytes indefinitely in man's environment. Man is exposed to the spores of these fungi by inhalation or by subcutaneous implantation on thorns or splinters. Ingestion of spores probably plays an insignificant role in most mycoses. The frequency with which man is exposed to spores of these fungi would be more alarming were it not that most cases of infection are mild and one infection apparently confers immunity against reinfection in the normal individual. The ubiquity of

fungi which are virulent or potentially pathogenic for man should concern every mycologist interested in soil microbiology and aerobiology.

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FUNGI FROM SALT LAKES. I. A NEW SPECIES OF CLAVARIOPSIS

C. J. Anastasiou

(with 2 figures)

In December, 1960, an undescribed species of *Clavariopsis* was found growing on the wood of *Tamarix aphylla* (L.) Karst. taken from a tree submerged in the Salton Sea, California. The organism was studied in the laboratory from material growing on the original substrate and in pure culture.

The problem of generic disposition of this fungus raised a basic taxonomic question. Saccardo (**13**) described the genus *Heliscus* in very broad terms, including all those forms bearing spores in which the main axes develop several divergent processes. Specifically, he stated: "*Conidia cylindracea, apice clavi ad instar polygono capitata. . .*" (**14**). This generic description easily could include *Clavariopsis,* which was described at a later date by De Wildeman (**3**). However, according to Ingold (**6**), one should not include *Clavariopsis* and *Heliscus* in the same genus simply on the basis of spore shape because *Clavariopsis* produces aleuriospores and *Heliscus* produces phialospores. The genus *Clavariopsis,* therefore, is emended to include only those forms, within existing generic limits, which are aleuriosporous. *Heliscus* is emended to include those forms, within existing generic limits, which are phialosporous.

Clavariopsis is a genus containing two described species, *C. aquatica* De Wildeman (**3**) and *C. brachycladia* Tubaki (**15**). Several other undescribed spore forms are possible members of the genus (**5, 7, 8**). All the above fungi have been isolated from fresh-water habitats.

Clavariopsis bulbosa Anastasiou, sp. nov.

Fungus aquaticus submersus. Mycelio septato, ramoso. Conidiophoris hyalinis, septatis, ramosis vel simplicibus, 10–300 × 3–5 μ. Aleuriosporis terminalibus, prima hyalinis, postea leviter olivaceis, septatis, ex quattuor bracchiis. Bracchio basale 1-septato, cellula proxima bulbosa, 10.5–(13.5)–20.0 × 6.2–(8.4)–10.0 μ; cellula distante 6.5–(8.4)–10.4 × 6.6–(9.8)–13.2 μ; bracchiis divergentibus 1–7–septato, 4–(18.7)–70 × 3.8–(5.6)–8.4 μ, ex cellula distante bracchii basalis. Chlamydosporis catenulatis prima atro-olivaceis, postea nigris. Catenis raro ramosis, ex 2–20 sporis; sporis basalibus fere longis, tenuibus, 9–(12.7)–18 × 4–(7.5)–10 μ aliis leviter longioribus quam latis, 10–(12.3)–18.2 × 7–(11.0)–14 μ.

Submerged aquatic fungus with branched, hyaline mycelium. Conidiophores, hyaline, septate, branching or unbranched, 10–300 μ in length, 3.5 μ in diam. Aleuriospores apical, hyaline, darkening slightly in age, septate, with four arms. Basal arm uniseptate, proximal cell bulbous, 10.5–(13.5)–20.0 × 6.2–(8.4)–10.0 μ; distal cell 6.5–(8.4)–10.4 × 6.6–(9.8)–13.2 μ; divergent arms 1–7–septate, 4–(18.7)–70 × 3.8–(5.6)–8.4 μ, arising from the distal cell of the basal arm. Catenulate chlamydospores deep olivaceous, black in age, 2–20 spores forming a rarely branched chain, basal spore usually long and thin, 9–(12.7)–18 × 4–(7.5)–10 μ; others slightly longer than broad, 10–(12.3)–18.2 × 7–(11.0)–14 μ.

HOLOTYPE: California, U.S.A. On athel wood (*Tamarix aphylla*) submerged in the southern marshes of the Salton Sea, Imperial County. Salinity, 23,000 ppm. RSA culture and herbarium specimen #19. Transfers of this holotype have been deposited in the A. T. C. C., C. B. S., and C. M. I.

Clavariopsis bulbosa is similar to the previously described species in both morphology and ontogeny. The septum locations are identical in each of the species. The basal arm regularly is uniseptate and is composed of two unequal cells. When the divergent arms become septate, the septa delimiting them from the basal arm are located at the points of origin of the divergent arms (FIG. 1g, p) (**3**, **15**). This is not true of other genera forming four-armed spores, e.g., *Lemonniera* (**9**, **15**).

Clavariopsis bulbosa is distinguished from the other species of the genus by the length and diameter of the divergent arms, conspicuously bulbous proximal cell of the basal arm, catenulate secondary spores, cultural characteristics, and habitat, among other less obvious features.

Pure culture was obtained on sea water agar (**10**) made up with Salton Sea water. The fungus also grows well on fresh water media [MeYe, YpSs, and corn meal agar (**2**)], but good sporulation was obtained only on YpSs and SSwa (Salton Sea water agar). The substitution of fresh water for Salton Sea water in SSwa did not result in sporulation. *Clavariopsis bulbosa* produces a blackish mouse gray culture with deep olive gray aerial mycelium. In reverse the culture appears deep blue-green gray with a blackish mouse gray border (**12**).

It is not surprising that *Clavariopsis bulbosa* grows well on fresh water media because several other investigators of halophilous fungi have reported similar results for other species (**1**, **10**, **11**). *C. bulbosa* is classified, therefore, as a facultative halophile (**11**).

Unlike other described species of *Clavariopsis*, *C. bulbosa* produces relatively normal spores aerially on agar media (FIG. 1 a, b, q–x). These

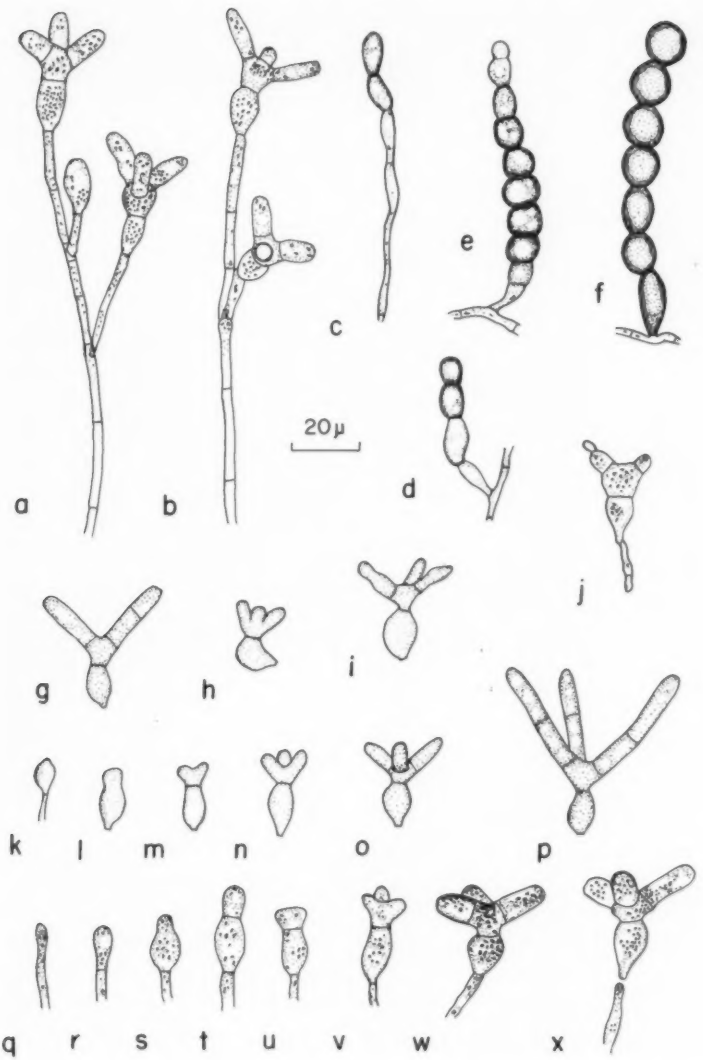

FIG. 1. *Clavariopsis bulbosa*. a–b. 4-armed aleuriospores on branching conidiophores (from culture); c–f. development of catenulate chlamydospores; g–i. malformed aleuriospores from athel wood; j. germinating immature aleuriospore; k–p. developmental stages of aleuriospores on submerged athel wood; q–x. developmental stages of aleuriospores aerially produced in culture.

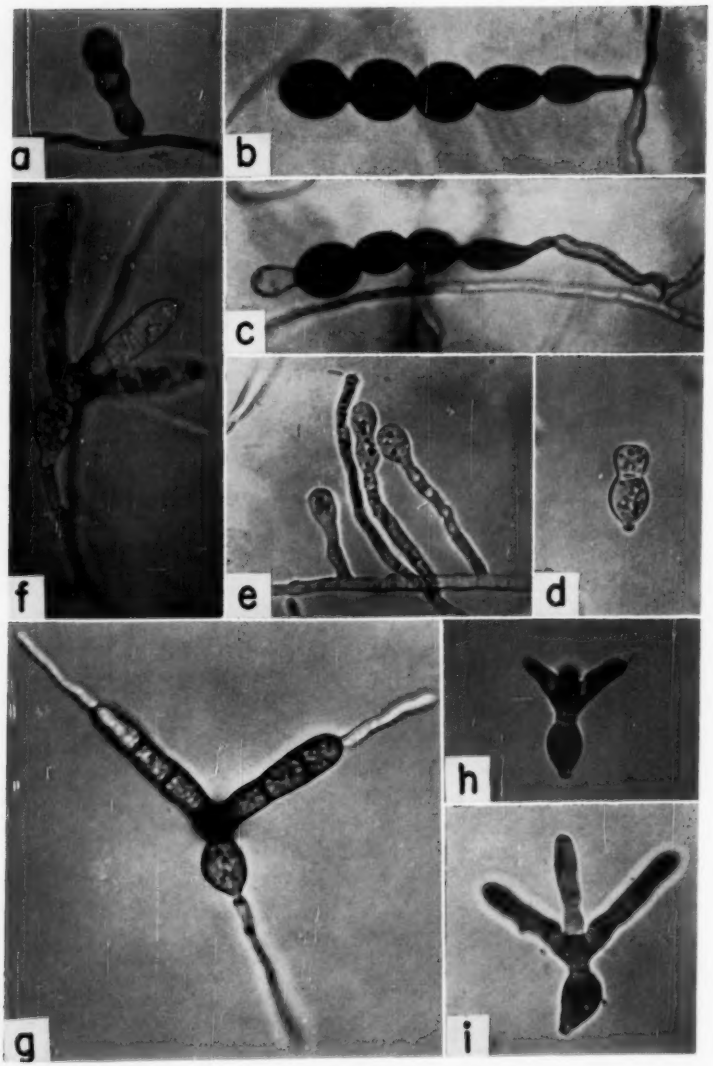

FIG. 2. *Clavariopsis bulbosa*. a. chlamydospore chain primordium; b. mature chlamydospore chain; c. mature chlamydospore chain budding an additional cell at the apex; d. dislodged immature aleuriospore (from culture); e. aleuriospore primordia with delimiting septa (from culture); f. mature aleuriospore with granular cytoplasm (from culture); g. mature aleuriospore with germination tubes; h-i. mature aleuriospores from athel wood. Magnification ×640.

spores differ slightly from those produced on athel wood immersed in Salton Sea water. The cytoplasm is much more granular in culture (FIG. 2, compare f, d and h, i). The main morphological difference lies in the diameter of the divergent arms. Those formed in culture are 4.2–(6.0)–8.4 μ; those growing immersed on the original substrate are 3.8–(5.2)–6.6 μ (FIG. 2, compare f, g and h, i). Development of the four-armed spores in culture (FIG. 1 q–x; FIG. 2 d–f) is similar to their development on the immersed wood sample (FIG. 1 k–p). A greater proportion of malformed spores occurred in culture, but they also occurred on the original immersed substrate (FIG. 1 g–i). Spores may be liberated before production of the divergent arms (FIG. 1 l; FIG. 2 d), but they usually remain attached until maturity (FIG. 2 f).

Aleuriospores germinate in Salton Sea water, sea water, or distilled water in 24 to 48 hours. Each spore cell is capable of germinating, but the thin germ tubes usually developed at the apices of the arms (FIG. 1 j; FIG. 2 g).

Catenulate chlamydospores are formed on lateral branches of the mycelium ramifying in the agar medium and in the lumens of wood cells in athel wood. These spores occasionally are produced from conidiophores bearing four-armed aleuriospores. The chains usually develop from 2–4-celled primordia (FIG. 1 d; FIG. 2 a), but occasionally the primordia are quite long (FIG. 1 c). The cells enlarge basipetally, with increasing vacuolation (FIG. 1 c, d). Their color deepens to dark olivaceous, and in age becomes almost opaque and black. During maturation, by division of maturing cells, additional cells may be added to the chain acropetally, basipetally, or in both directions (FIG. 1 e). After maturation, additional cells may be added acropetally by a striking process of budding (FIG. 2 c). In old cultures the entire submerged mycelium may be converted into this type of spore.

Catenulate chlamydospores have been reported for other aquatic fungi. *Lulworthia salina* (Linder) Cribb & Cribb and *Peritrichospora integra* Linder are said to produce this type of spore in culture (**1**). Describing them as "thick walled cells," Tubaki (**15**) reports similar structures for *Dactylella microaquatica* Tubaki.

Clavariopsis bulbosa appears to be the first halophilous representative of a genus which previously included only fresh water aquatic Hyphomycetes. This may serve as an indication of a relationship between the salt lake fungus flora and the fresh water fungus flora. For many years after its formation in 1904–5, the Salton Sea was a fresh water lake (**4**). It is possible that *C. bulbosa* is a fresh water species capable of flourishing under the conditions of high salt concentration which developed in the

Salton Sea. On the other hand, it is possible that *C. bulbosa* originated in the oceans along with many other species which will be reported in future papers of this series.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. R. K. Benjamin for his guidance and helpful criticism throughout the preparation of this paper, to Dr. P. A. Munz for help with the Latin diagnosis, and to Mr. K. Wagner, who assisted with the original collection and carried out salinity determinations. The author is also indebted to Dr. C. T. Ingold for helpful suggestions and to Dr. F. V. Ranzoni who provided cultures for comparison.

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NORTH AMERICAN SPECIES OF PUCCINIA ON HYPTIS

JOHN W. BAXTER[1]

(WITH 7 FIGURES)

This paper reviews the species of *Puccinia* parasitizing hosts of the genus *Hyptis* in North America. It is a continuation of a taxonomic study of the rust fungi occurring on members of the family Labiatae throughout the world. Baxter and Cummins in 1951 (**3**), Baxter in 1953 (**1**), and Baxter in 1955 (**2**) presented taxonomic treatments of the species of *Puccinia* known to occur on *Salvia*.

In the present study considerable emphasis is placed on urediospore characters in separating species in the key. In discussing the species having urediospores with two equatorial or subequatorial pores, use is made of the system of urediospore types described and illustrated by Baxter and Cummins (**3**) in their treatment of the North American *Salvia* rusts. In some of the species on *Hyptis*, as in many of the *Salvia* rusts, the urediospores are radially asymmetrical. In such species the diameter and shape of an individual urediospore depend upon whether it is viewed with the pores in surface view or with the pores in lateral view.

Uredia present; teliospore wall smooth
- Urediospore pores equatorial
  - Urediospore pores 3 ..... 1. *P. insititia*
  - Urediospore pores 2
    - Teliospore wall umbonate at apex ..... 2. *P. hyptidis-mutabilis*
    - Teliospore wall uniform at apex
      - Teliospores 45–84 μ long ..... 3. *P. hyptidis*
      - Teliospores 30–40 μ long ..... 4. *P. medellinensis*
- Urediospore pores subequatorial, near the hilum
  - Urediospore pores 1 to 3 ..... 5. *P. parilis*
  - Urediospore pores 4 or 5 ..... 6. *P. fidelis*

Uredia lacking; teliospore wall verrucose ..... 7. *P. distorta*

[1] The writer acknowledges with appreciation the loan of specimens by the Institute of Systematic Botany, University of Uppsala, Uppsala, Sweden and the Arthur Herbarium, Purdue University. This study was supported by a grant from the National Science Foundation.

1. PUCCINIA INSITITIA Arth., Mycologia 7: 248. 1915. FIG. 1
Dicaeoma insititium (Arth.) Arth., N. Am. Flora 7: 409. 1921.

Spermagonia and aecia unknown. Uredia hypophyllous, cinnamon-brown, urediospores broadly ellipsoid or globoid, 20–25 × 22–29 μ; wall cinnamon-brown or yellowish, 1–1.5 μ, closely and finely verrucose-echinulate, pores 3, equatorial. Teliospores in the uredia; narrowly ellipsoid or clavate, 16–24 × 48–55 μ, wall cinnamon-brown, yellowish or occasionally hyaline, about 1 μ, thickened to 2–3 μ and paler at the apex, smooth; pore of upper cell apical, of lower next to the septum; pedicel thin-walled, hyaline, 30 μ or less in length.

HOSTS AND DISTRIBUTION: *Hyptis lantanifolia* Poit., W. Indies, Brazil; *Hyptis atrorubens* Poit., W. Indies.

TYPE SPECIMEN: On *Hyptis lantanifolia*, Manáos, Brazil, 1901, *Ule 7*.

The sculpturing of the urediospore wall and the presence of three equatorial pores are the distinctive characteristics of this species.

2. PUCCINIA HYPTIDIS-MUTABILIS Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 496. 1913. FIG. 2

Dicaeoma Hyptidis-mutabilis (Mayor) Arth., N. Am. Flora 7: 410. 1921.

Eriosporangium hyptidis-mutabilis (Mayor) Syd., Ann. Myc. 20: 122. 1922.

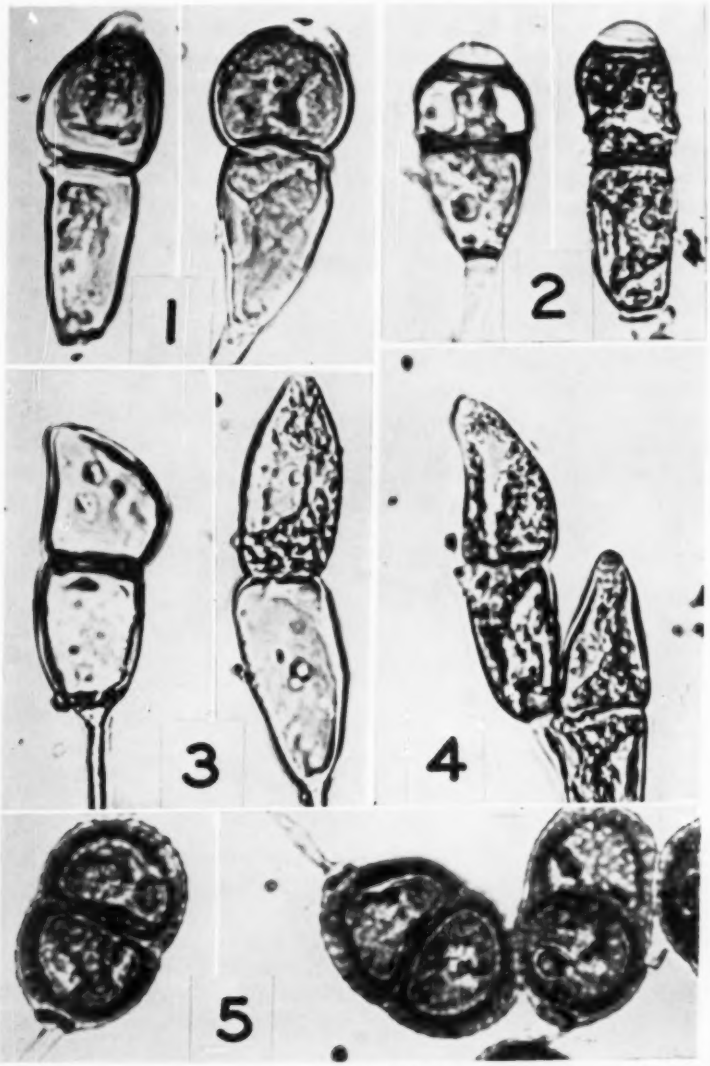
Uredo amphiospora Jacks. & Holw. in Jacks., Mycologia 24: 72. 1932.

Puccinia amphiospora Cumm., Bull. Torr. Bot. Club 67: 67. 1940.

Spermagonia not seen. Aecia amphigenous, aecidioid; peridium short, fragile; aeciospores globoid or ellipsoid, 16–19 × 20–30 μ; wall yellowish, 1.5–2.5 μ, verrucose. Uredia hypophyllous, cinnamon-brown; urediospores oblate-spheroid, 23–26 μ broad × 17–23 μ high, or globoid and 20–23 μ in diameter; wall cinnamon-brown or yellowish, 1–2 μ, echinulate, pores 2, equatorial. Amphisori amphigenous, chiefly hypophyllous, chestnut-brown, amphispores broadly ellipsoid or globoid, 20–25 × 25–29 μ; wall chestnut- or cinnamon-brown, 2–3(–4) μ, obscurely echinulate or frequently nearly smooth, pores 2, equatorial. Telia hypophyllous, cinnamon-brown or light chestnut-brown; teliospores cylindrical, clavate or narrowly ellipsoid, 16–20 × 27–57 μ; wall yellow, 1 μ thick, smooth; pore of upper cell apical, of lower next to the septum; each capped by a hyaline umbo 2–5 μ thick; pedicel thin-walled, hyaline, up to 25 μ long.

HOSTS AND DISTRIBUTION: *Hyptis dubia* Pohl, S. America; *H. mutabilis* (Rich.) Briq., Florida, Guatemala, El Salvador, Honduras, Costa

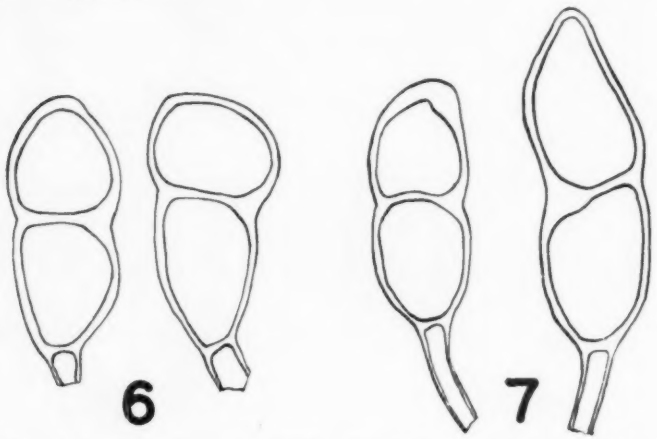
Rica, W. Indies, S. America; *H. pectinata* (L.) Poit., Florida, Guatemala, S. America, Africa; *H. spicata* Poit., S. America; *H. suaveolens* (L.) Poit., S. America; *H. umbrosa* Selzm., S. America.



FIGS. 1–5. Teliospores. 1. *Puccinia insititia* (from Dale *551*), ×900. 2. *P. hyptidis-mutabilis* (from Holway *1368*), ×900. 3. *P. hyptidis* (from type), ×900. 4. *P. fidelis* (from type), ×900. 5. *P. distorta* (from type), ×900.

TYPE SPECIMEN: On *Hyptis mutabilis*, El Banco, Dept. Magdalena, Colombia, July 27, 1910, *Mayor 121*.

The urediospores of this species are mostly of Baxter and Cummins' Type 3, being oblate-spheroid when viewed with the pores in lateral view and globoid when viewed with the pores in surface view. Occasional urediospores are radially symmetrical and are globoid in both views. The amphispores are of Type 1, being narrower when viewed with the pores in lateral view. They may occur in the uredia or in larger, darker amphisori. In uredia containing both urediospores and amphispores, it is not unusual to find a considerable number of spores that are intermediate in appearance.



FIGS. 6, 7. Teliospores. 6. *Puccinia medellinensis* (from type), ×900. 7. *P. parilis* (from type), ×900.

A recent paper by Jørstad (**4**) reports two collections of *P. hyptidis-mutabilis* from Africa. Deighton's collection CB905 on *H. pectinata* from the Gold Coast, previously assigned to *Puccinia medellinensis* Mayor, is probably *P. hyptidis-mutabilis*.

3. PUCCINIA HYPTIDIS (Lagerh.) Tracy & Earle, Bull. Miss. Exp. Sta. **34**: 86. 1895. FIG. 3

Uredo hyptidis Curt., Am. Jour. Sci II. **6**: 353. 1848.

Gymnoconia hyptidis Lagerh., Tromso Mus. Aarsh. **17**: 83. 1895.

Argotelium hyptidis (Lagerh.) Arth., Res. Sci. Congr. Bot. Vienne: 343. 1906.

Eriosporangium hyptidis (Lagerh.) Arth., N. Am. Flora **7**: 211. 1912.

Dicaeoma hyptidis (Lagerh.) Arth., N. Am. Flora **7**: 408. 1921.

Spermagonia amphigenous. Aecia amphigenous, caeomoid; aeciospores ellipsoid or globoid, 20–32 × 27–40 μ, wall hyaline, 2–3 μ, coarsely verrucose. Uredia hypophyllous, cinnamon-brown; urediospores broadly ellipsoid or globoid, 20–26 × 23–30 μ, wall cinnamon-brown, 1–2 μ, echinulate, pores 2, equatorial. Telia hypophyllous, cinnamon-brown or yellowish; teliospores cylindrical, fusiform or clavate, 13–20 × 45–84 μ; wall yellow or hyaline, about 1 μ thick, smooth; pore of upper cell apical, of lower next to the septum; pedicel thin-walled, hyaline, persistent, up to 95 μ long.

HOSTS AND DISTRIBUTION: *Hyptis canescens* Benth., S. America; *H. capitata* Jacq., W. Indies; *H. spicigera* Lam., Philippines; *H. conferta* Pohl, Honduras; *H. radiata* Willd., Florida, Alabama, Mississippi, Texas; *H. shaferi* Britton, Cuba.

TYPE SPECIMEN: On *Hyptis radiata*, Alabama, *Curtis*.

The treatment of this species by Tracy and Earle (**6**) was merely a transfer and was not accompanied by a description of the perfect state. The first valid description of the telial stage is that by Lagerheim (**5**) under the name *Gymnoconia hyptidis*. Sydow (**7**), under the name *Puccinia hyptidis* (Curt.) Tracy and Earle, actually redescribed *Puccinia gibertii* Speg.

4. PUCCINIA MEDELLINENSIS Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 497. 1913. FIG. 6

Uredo hyptidis-atrorubentis Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 593. 1913.

Dicaeoma medellinense (Mayor) Arth., N. Am. Flora **7**: 408. 1921.

Eriosporangium medellinense (Mayor) Syd., Ann. Myc. **20**: 122. 1922.

Spermagonia amphigenous. Aecia amphigenous and caulicolous, aecidioid; peridium short, fragile; aeciospores ellipsoid or globoid, 16–24 × 23–34 μ; wall hyaline, 1.5–2.5 μ, verrucose. Uredia hypophyllous, cinnamon-brown; urediospores oblate-spheroid with pores in lateral view and globoid with pores in surface view, 12–22 × 19–23 μ; wall cinnamon-brown, 1–1.5 μ, echinulate, pores 2, equatorial. Telia hypophyllous, pale yellow to cinnamon-brown, compact; teliospores narrowly ellipsoid, 13–20 × 30–40 μ; wall pale yellow or hyaline, 1 μ thick, smooth, pore of upper cell apical, of lower next to the septum; pedicel thin-walled, hyaline, up to 50 μ long.

HOSTS AND DISTRIBUTION: *Hyptis atrorubens* Poit., S. America, W. Indies; *H. brevipes* Poit., Guatemala, Honduras; *H. capitata* Jacq., Guatemala; *H. pectinata* (L.) Poit., Guatemala, Costa Rica, W. Indies,

S. America; *H. polystachya* H.B.K., Guatemala, Costa Rica, El Salvador; *H. suaveolens* (L.) Poit., Guatemala, Nicaragua, Costa Rica, Honduras, S. America; *Hyptis* sp., Costa Rica.

TYPE SPECIMEN: On *Hyptis pectinata*, Medellin, Colombia, Aug. 26, 1910, *Mayor 295 d.*

Puccinia medellinensis resembles *Puccinia perfuncta* Jacks., a South American species, but differs in having longer teliospores and darker, thicker-walled urediospores.

Aecidium hyptidis P. Henn., listed by Sydow (7) as a synonym of *P. medellinensis*, probably belongs to *P. perfuncta* or *P. gibertii*.

5. PUCCINIA PARILIS (Arth.) Arth., Am. Jour. Bot. **5**: 485. 1918. FIG. 7

Argomyces parilis Arth., N. Am. Flora **7**: 217. 1912.
Bullaria parilis (Arth.) Arth. & Mains, N. Am. Flora **7**: 494. 1922.

Spermagonia and aecia uncertain. Uredia hypophyllous, cinnamon- or chestnut-brown; urediospores oblate-spheroid, 20–23 μ broad × 14–20 μ high, or globoid, 14–23 μ in diameter; wall cinnamon-brown, 1.5–2 μ, echinulate, pores 2 or 3, subequatorial. Amphispores in the uredia, broadly ellipsoid, obovoid or globoid, 16–22 × 16–24 μ; wall 2–3(–4) μ, closely verrucose echinulate, pores 2, occasionally 1, near the hilum. Telia hypophyllous, white or pale yellow, compact or somewhat pulverulent; teliospores narrowly ellipsoid, oblong or fusiform, 16–20 × 33–50 μ, wall hyaline, about 1 μ thick, smooth, pore of upper cell apical, of lower next to the septum; pedicel thin-walled, hyaline, up to 50 μ long.

HOSTS AND DISTRIBUTION: *Hyptis pectinata* Poit., Mexico; *H. stellulata* Benth., Guatemala.

TYPE SPECIMEN: On *Hyptis pectinata*, Oaxaca, Mexico, Nov. 15, 1903, *Holway 5419.*

It is possible that this species has uredinoid aecia. Spermagonia are present in the type specimen, but are not closely associated with the uredia. The uredia are scattered and not grouped as uredinoid aecia would be. Elucidation of the complete life cycle of *P. parilis* must await collection of additional material.

As in *P. hyptidis-mutabilis*, there is intergradation between amphispores and urediospores.

6. PUCCINIA FIDELIS Arth., Bull. Torr. Bot. Club **38**: 369. 1911. FIG. 4

Eriosporangium fidelis (Arth.) Arth., N. Am. Flora **7**: 212. 1912.
Dicaeoma fidele (Arth.) Arth., N. Am. Flora **7**: 409. 1921.

Spermagonia hypophyllous. Aecia caeomoid, hypophyllous and caulicolous, causing hypertrophy; aeciospores globoid, 20–24 × 23–30 μ, wall hyaline, 2–3 μ thick, coarsely verrucose. Uredia hypophyllous, cinnamon-brown; urediospores globoid, 20–24 × 23–26 μ; wall cinnamon-brown, 1.5–2 μ, closely verrucose-echinulate; pores 4 or 5, near the hilum. Telia hypophyllous, cinnamon-brown, yellow or nearly white, compact; teliospores fusiform or cylindrical, 13–20 × 43–68 μ; wall hyaline, 1 μ or less, smooth, pore of upper cell apical, of lower next to the septum; pedicel thin-walled, hyaline, up to 70 μ long.

HOSTS AND DISTRIBUTION: *Hyptis lilacina* (Schiede and Deppe) Kuntze, Guatemala, Costa Rica; *H. oblongifolia* Benth., Guatemala; *H. pectinata* (L.) Poit., Guatemala; *H. stellulata* Benth., Mexico; *H. urticoides* H.B.K., Guatemala.

TYPE SPECIMEN: On *Hyptis stellulata,* Guadalajara, Mexico, July 21, 1893, *Pringle 33.*

The presence of four or five basal pores in the urediospore serves to distinguish *P. fidelis* from other species of *Puccinia* on *Hyptis.*

7. PUCCINIA DISTORTA Holw., Ann. Myc. **3**: 22. 1905. FIG. 5

Dasyspora distorta (Holw.) Arth., Res. Sci. Congr. Bot. Vienne: 346. 1906.

Micropuccinia distorta (Holw.) Arth. and Jacks. in Arth., Bull. Torr. Bot. Club **48**: 40. 1921.

Spermagonia epiphyllous. Aecia and uredia lacking. Telia amphigenous and caulicolous, causing hypertrophy on stems, chocolate- or chestnut-brown, pulverulent; teliospores broadly ellipsoid, 20–26 × 30–40 μ; wall chestnut-brown, 2–4 μ, finely verrucose, pore of upper cell apical, of lower midway between septum and pedicel; pedicel thin-walled, hyaline, deciduous or occasionally persistent and up to 35 μ long.

HOSTS AND DISTRIBUTION: *Hyptis emoryi* Torr., California, Arizona; *H. pectinata* Poit., Mexico; *H. stellulata* Benth., Guatemala; *Hyptis* spp., Mexico, S. America.

TYPE SPECIMEN: On *Hyptis pectinata,* Iguala, Guerrero, Mexico, Nov. 3, 1903, *Holway 5323.*

Puccinia distorta is the only microcyclic rust known to occur on *Hyptis.*

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THE GENUS SCHENELLA [1]

G. W. Martin

(with 4 figures)

The genus *Schenella*, with its single species, *S. simplex*, was described by Macbride (1911) on the basis of a single specimen collected on a pine log in Yosemite National Park, California, in 1903. Macbride was not sure it was a myxomycete, but stated that if it was, it should be referred to the Dianemaceae. The description was accompanied by seven diagrammatic figures, which give what appears to be an accurate impression of its original appearance. Macbride did not mention the species in the second edition of North American Slime-Moulds (1922), nor was it included in the third edition of the Lister monograph (1925). Macbride and Martin (1934) placed it in the Stemonitaceae; Hagelstein (1944) made casual mention of it under the Amaurochaetaceae, a family which seems unworthy of recognition, and Martin (1949), in a similar note, suggested that if valid it should be placed in the Stemonitaceae near *Amaurochaete*.

The type collection is in the herbarium of the University of Iowa in the two small boxes in which it was originally placed. Because of its extreme fragility it has not been removed from these. So far as the material permits comparison with the original description and the accompanying drawings the facts as given there are essentially correct. They do need reinterpretation.

I believe the fructification should be regarded as a pseudoaethalium, with the sporangia arranged much as in *Dictydiaethalium* or in the early stages of *Tubifera ferruginosa* (Batsch) J. F. Gmel. The cortex was evidently originally continuous, as shown in Macbride's figures 1 and 2. The columns appear to represent individual sporangia, each with at least a partial and probably an originally complete peridium. A partial peridium is shown in Macbride's figure 4. Such structures still exist at the bases and apices of the sporangia and fragments of similar nature are interspersed amongst the capillitium and spores, constituting a very crude sort of pseudocapillitium. There is no trace of a columella, but the bundles of loosely coiled, unbranched capillitial threads remain united

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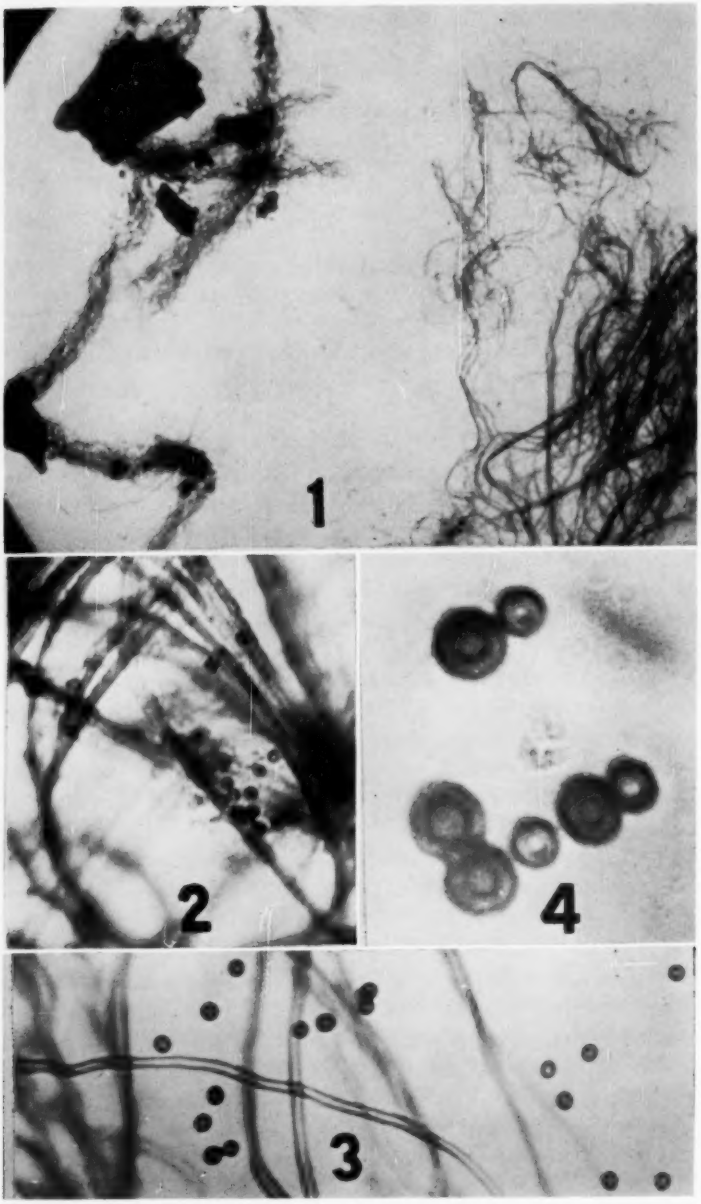

FIGS. 1-4.

in their original groups, each bundle inserted in an inverted conical base surrounded by a peridium there and again at the apex, where the black cap, often with peridial remnants attached, is united to the cortex in such a way that it seems clear that each represents the apex of a sporangium and that the coiled threads in each column represent the capillitial system of such a sporangium. This is shown accurately in Macbride's figure 6. The dark patch in the upper left-hand corner of FIG. 1 is such a fragment of the cortex bearing several caps, the whole appearing dark because of adherent spores; five capillitial columns may be seen attached to it. In the lower left-hand corner the dark bodies are those of two basal cups from which two capillitial columns arise, one of which is also attached at the apex. The dark body half-way between the basal cups and the figure is a fragment of peridium, apparently detached from a sporangial wall. The spores and capillitium are nearly black in mass with a slight suggestion of fuscous, about Blackish Brown 1 of Ridgway. By transmitted light, both spores and capillitium, are brown, without any suggestion of purple. The spores are distinctly, somewhat irregularly, warted. Macbride says "smooth," but his figure 6 shows a strongly tuberculate spore and the magnification given should be reduced to ×2000. The spores are small for a myxomycete, but no smaller than in other well-known species. I am unable to find any suggestion that they have been formed in any way other than is the case in Myxomycetes.

The lack of columella and the attachment of the capillitial threads to the base and cortex do suggest the Dianemaceae, as stated by Macbride, but I am inclined to stress the dark spores and capillitium and the obvious general similarity to *Amaurochaete* and to assign the genus to the Stemonitaceae. On the basis of these observations, I append an emended description of the genus and its original species.

SCHENELLA Macbride, Mycologia **3**: 39. 1911.

Fructification a pseudoaethalium, expanded, depressed, covered by a fragile continuous cortex. Sporangia columnar, erect, each with constricted base and persistent lid which is firmly attached to the cortex, the peridium tending to be persistent at the apex and at the base, otherwise fugaceous. Columella none. Capillitium of numerous equal, tortuous, unbranched threads attached at base and to lid and more or less coiled into columnar strands. Spores black in mass.

FIGS. 1–4. *Schenella*. 1. *S. simplex* (left) and *S. microspora* (right) showing difference in size of capillitial columns, ×12½. 2. *S. microspora*, rough capillitium and spores, ×ca. 300. 3. *S. simplex*, smooth capillitium and spores, ×ca. 300. 4. Spores of both species mounted together, ×ca. 2000.

Schenella simplex Macbride, Mycologia **3**: 39. 1911.

Pseudoaethalium oval in outline, expanded, depressed, attaining a length of 4 cm, a width of 2 cm, and 3 mm in thickness; cortex continuous, with minute bosses indicating position of tops of the sporangia, pallid or gray, somewhat shining; sporangia erect, columnar, attached at bases and tips where peridium, elsewhere fugaceous, persists; capillitium black in mass, dark brown under lens, profuse, of sinuous, unbranched, nearly smooth threads mostly 3–4 μ in diameter, remaining united and coiled into columnar strands; spores globose, black in mass, bright yellowish brown by transmitted light, distinctly verrucose, 5–6 μ in diameter.

Known only from the type collection.

At the A.I.B.S. meeting at Stanford University in 1957, an excursion was made to the Big Basin State Park in San Mateo County. There, on a large fallen decorticated trunk of coast redwood, I found a cluster of three closely associated apparent aethalia, which I took to represent an *Amaurochaete*. The trunk was hard, the specimens fragile and my knife dull, so the collection is badly shattered. Upon return to my laboratory I reexamined it and after noting its unusually small spores, put it aside as an undescribed *Amaurochaete*. After reexamining *S. simplex*, I recalled this collection and examined it again. It is unquestionably a *Schenella*, but differs from *S. simplex* in several respects. The color of the spore-mass is distinctly vinaceous, about Dark Vinaceous Drab of Ridgway. The capillitial threads are coiled in columns but less distinctly so than in *S. simplex*, although some are united, and the columns are much more slender. They are similarly attached at the base. The chief difference, however, is in the spores, which are smoother and much smaller than those of *S. simplex*, mostly 3.5–4 μ in diameter, and much paler in color under the lens. There were remnants of a gray cortex on the wood when it was found, but only minute fragments survive. The capillitial threads are somewhat more variable in size and distinctly roughened.

I should hesitate in most cases to describe a new species on the basis of so old and shattered a collection even though it is better than most of our Amaurochaetes, but since I am sure it is a *Schenella* and not an *Amaurochaete*, since it differs from *S. simplex* in color, capillitium and spores, and doubtless in other characters, and especially since *Schenella* has been regarded as a doubtful genus since its establishment, I venture to give it a name in the hope that others who have opportunity to collect in the western mountains will watch for additional examples.

Schenella microspora Martin, sp. nov.

Pseudoaethalium pulvinatum, corticatum, 4 cm longitudine, 1 cm latitudine attingens; cortice fragile, pallida; sporangiis tenuis, peridiis evanescentibus, capillitio et sporis atro-vinaceis in massa; filamentibus sinuosis, circinatis, simplicibus, in racemis basi et cortice adhaesis aggregatis; filamentis asperis, 2–6 μ diam.; sporis brunneis, minute verrucosis, 3.5–4(–4.5) μ diam. Plasmodium ignotum.

Pseudoaethalium pulvinate, corticate, up to 4 cm in length, 1 cm wide and about 5 mm thick after disappearance of the cortex; cortex fragile, pale, early breaking away; sporangia slender, the peridium disappearing early except for remnants at top and bottom of capillitial clusters; capillitium and spores Dark Vinaceous Drab (R) in mass; capillitium united into columnar strands, each strand representing the capillitium of a constituent sporangium, the threads brown under the lens, distinctly roughened, 2–6 μ in diameter; spores globose, minutely verrucose, bright yellow brown under lens, 3.5–4(–4.5) μ in diameter. Plasmodium unknown.

CALIFORNIA: Big Basin State Park, San Mateo County, 26 August 1957, on fallen trunk of *Sequoia sempervirens* (Lamb.) Endl. *G. W. Martin 6547*, TYPE (IA).

It seems obvious that *Schenella* is closely related to *Amaurochaete*, from which it differs mainly in the unbranched, coiled, uniform capillitial threads united into columns and with no trace of a columella or branches. The unusually small spores are distinctive, but do not, of themselves, warrant generic segregation. The fructification of *Amaurochaete* is commonly referred to as an aethalium. In all four species recognized by Martin (**1949**), there is evidence that they are composed of sporangium-like units and the capillitium usually arises from stout columella-like bases, branching and anastomosing much in the manner of that of a *Stemonitis*. This is so striking that Hagelstein (**1944**) referred *A. trechispora* Macbr. & Martin to *S. trechispora* (Torrend) Macbr. and *A. ferruginea* Macbr. & Martin to *S. splendens* Rost. var. *flaccida* List. Without agreeing with this disposition, it is plain that the suggestion of constituent sporangia is present in these species and in the other two, *A. Tubulina* (Alb. & Schw.) Macbr. and *A. fuliginosa* (Sow.) Macbr. It is less apparent in *A. comata* G. List. & Brândză, which I know only from G. Lister's description and illustration (**1926**, p. 225, *pl. 578*) and from a very greatly weathered specimen collected by Brândză in the type locality and distributed as Myxom. Roum. *95*, but even in that species there is some evidence of basal stalks from which the capillitium arises and its abundant branches and anastomoses constitute a capillitial system basically distinct from that of *Schenella*.

It is quite possible that the fructifications of all species of *Amaurochaete* should be classed as pseudoaethalia. Unfortunately, most specimens, as collected, are so old that it is difficult to make sure of this point, but it is quite apparent in some of them.

SUMMARY

Reexamination of the type and only known collection of *Schenella simplex* Macbride leads to the conclusion that *Schenella* is a distinct genus of the Myxomycetes, most closely allied to *Amaurochaete* and to be included in the Stemonitaceae. A second species is described, *S. microspora*, also from California.

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SOME FUNGAL PARASITES OF LILIACEAE [1]

D. B. O. Savile

The following notes present one new species, four transfers, and emended descriptions, new hosts or range extensions of various parasitic fungi that attack members of Liliaceae in the broad sense. Most of the records are Canadian, especially from British Columbia, but some other records are included, particularly for the rusts of *Allium*.

To save space in citation of specimens, collectors' names are abbreviated thus: J. A. Calder (C), J. M. Ferguson (F), J. A. Parmelee (P), D. B. O. Savile (S), and R. L. Taylor (T). These names occur in various combinations. Specimens that lack a collector's number are cited by DAOM accession number. All specimens are in DAOM unless otherwise noted.

Ascomycetes

Botryotinia fuckeliana (de Bary) Whetz., Mycologia **37**: 679. 1945.

The conidial state is occasionally heavy on various Liliaceae. It is occasionally severe on *Convallaria majalis* L. at Ottawa, Ont., in crowded and shady beds (DAOM *15333, 15334*). We have it on this host from New Westminster, B.C. (DAOM *19945*). A single blighted blossom of *Fritillaria kamtschatcensis* (L.) Ker, apparently a new host, was found near Prince Rupert, B.C. (C, S & F *13172A*). Other records are on *Lilium* spp. (cult.), Ottawa, Ont., (DAOM *15498*) and Burnaby, B.C. (DAOM *26218*); and on *Uvularia perfoliata* L., Kingsmere, Gatineau Co., Que. (DAOM *26210*).

Ustilaginales

Tuburcinia clintoniae Komarov, F. Ross. Exs. *260*. 1899.

On *Streptopus roseus* Michx. var. *curvipes* (Vail) Fassett, at 6900 ft between Baldy Mtn. and Dunn Peak, near Littlefort, B.C. (C, P & T *19883*). Holway's collection from Glacier (F. Columb. *1789*), the only

[1] Contribution No. 160 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

other collection from British Columbia, is probably on the same host. Spore balls 34–102 × 30–72 μ, with from 10 to probably ca.100 spores.

UROCYSTIS CEPULAE Frost in Farl., Ann. Rep. Sec. Mass. Board Agr. **24**: 175. 1877.

Recorded by Fischer (**1953**), sub. *Urocystis colchici*, on *Allium cepa* L. from Alta., Man., Ont. and Que. We have since received a specimen from B.C.: Kelowna, P. G. James (DAOM *26068*). This specimen and material from eastern Canada yield spore balls with 1(–2) spores, (16–)-18–28(–35) × 14–24(–26) μ; spores 12.5–17(–19) × 11–15 μ, with wall ca. ?0.8 μ thick, chestnut; cortical cells nearly fully investing spores, 2.5–5(–7) μ high and 3.6–8 μ long, with wall generally ca. 0.3–0.5 μ thick, yellow-brown. I agree with Chupp (**1960**) in maintaining *U. cepulae* as a species distinct from *U. colchici*. The cortical cells of *U. cepulae* are often rounded, rather than flat as in Chupp's illustration; but they are smaller and thinner-walled than those of *U. colchici* (q.v.).

UROCYSTIS COLCHICI (Schlecht.) Rabenh., F. Europ. 396. 1861.

A specimen on *Colchicum autumnale* L. from near Sidney, Vancouver I., B.C., W. R. Orchard (DAOM *59519*) is apparently the first western collection on this host. Spore balls and spores agree closely with those in specimens from Ontario and Europe on this host: spore balls 20–57 × 16–37 μ, with 1–5(–8) spores; spores 12.5–21(–22.5) × 10–15.5 μ with wall ?0.5–1.0 μ thick, chestnut; cortical cells investing most of spore surface, 3.5–8.5 μ high and 4.5–12(–13.5) μ long, wall 0.5–1.5 μ thick, yellow-brown. One collection on *Camassia quamash* (Pursh) Greene, Oak Bay, Victoria, B.C. (C, P & T *16310*) agrees well; spore balls 19–53 × 15–36 μ, with 1–6 spores; spores 12.5–21 × 11–15 μ. Recorded on this host by Fischer (**1953**) from Oregon, and evidently to be sought in coastal Washington.

UROCYSTIS ERYTHRONII G. P. Clint. in Zundel, N. Am. Fl. 7(14): 1015. 1939.

Evidently more widespread on *Erythronium americanum* Ker than the records suggest. Since we discovered it at Ottawa in 1943, and learned to distinguish it in the field from *Ustilago heufleri*, we have found it at seven locations in Grey, Bruce, Lanark and Carleton Co., Ont., and Gatineau and Vaudreuil Co., Que. The sori are nearly linear and less conspicuous than those of *Ustilago heufleri*. It is only immature sori of the latter species that are readily confused with this smut.

USTILAGO HEUFLERI Fckl., Symb. Myc., 39. 1870.

Common in southern Quebec and southeastern Ontario (8 counties) on *Erythronium americanum* Ker, and probably nearly throughout the range of this host; but not previously recorded from western North America. On *Erythronium oregonum* Appleg. (*E. giganteum* auct. non Lindl., *E. grandiflorum* Pursh var. *albiflorum* Hook.), 2100 ft, start of trail to Forbidden Plateau, Vancouver I., B.C. (C, P & T *16474*). Should be sought in the humid coastal regions of Oregon and Washington. The smut is a good match for eastern material.

UREDINALES

PUCCINIA ATROPUNCTA Peck & G. W. Clint. in Peck, Bot. Gaz. **4**: 171. 1879.

A collection on *Zygadenus glaucus* Nutt. (*Z. chloranthus* Auct.), from Baie Ste. Claire, Gaspé Pen., Que., J. Adams (DAOM *2086*) gives a substantial northeastward range extension.

PUCCINIA GRUMOSA Syd. & Holw. in Syd., Monogr. Ured. **1**: 641. 1903.

On *Zygadenus elegans* Pursh, 24 miles W of West Dawson, Yukon (J. A. Calder & L. G. Billard *4515*); 5500 ft, slope of Mt. Stephen, near Field, B.C. (C & S *12062*); Banff, Alta., 25 July 1901 (DAOM s.n.), probably collected by Holway.

UROMYCES ZYGADENI Peck, Bot. Gaz. **6**: 239. 1881.

On *Zygadenus elegans* Pursh, 7 miles NE of Carey, Blaine Co., Ida., (C, S & T *20640*). On *Z. venenosus* S. Wats., B.C., Port Alberni, Vancouver I. (C, P & T *16429*), between Williams Lake and Quesnel (C, P & T *17003*), 19 miles N of Clinton (C, P & T *16642*), 4 miles W of Alexis Creek (C, P & T *17344, 18304*), 5 miles WSW of Kamloops (C & S *8523*), near Montana border, 5 miles N of Gateway (C & S *9195*). Idaho: 11 miles SE of Boise (C, S & T *20665*).

PUCCINIA VERATRI Duby, Bot. Gall. **2**: 890. 1830.

On *Epilobium latifolium* L., Palmer Creek Valley, Kenai Pen., Alaska (C *6258*) (*Veratrum eschscholtzii* common in area but rust not noted on it); Wall L., Waterton Lake Nat. Park, Alta. (T, F & C *3485*). On *E. luteum* Pursh (C, S & F *13322*) and on *Veratrum eschscholtzii* A. Gray (C, S & F *14863A*), 3200 ft, Mt. Thornhill, near Terrace, B.C. On *V.*

eschscholtzii A. Gray: Western Uranium Mine, 4500 ft, near Hazelton, B.C. (C, S & F *15202*); 2000 ft, Shields Bay, Graham I. (C & T *23366*), 400–2000 ft, Tasu Sound, Moresby I. (C & T *23707*), all Queen Charlotte Is., B.C. No associated aecia were secured in the Queen Charlotte Is., because access to the alpine and subalpine slopes was too difficult to warrant two visits to any mountain. From the heavy infection on *Veratrum* it is clear that the perpetually moist mountain slopes offer ideal conditions for this rust.

UROMYCES HOLWAYI Lagerh., Hedwigia **28**: 108. 1889.

The description of the aecia given by Arthur (**1934**) is so misleading that it must be based upon aecia of *Puccinia sporoboli* or some other grass rust. We have much material of this rust from the southern half of British Columbia on *Lilium columbianum*, including six collections of aecia, four of which are associated with uredinia or telia, or both, and two of which have incipient telia in the aecial sori. Identical aecia occur on single specimens of *L. canadense* (Ont.), *L. pardalinum* (Calif.), *L. parviflorum* (B.C.) and *L. philadelphicum* (Ill.), the second and third being accompanied by uredinia and telia, and telia being known from the site of the first specimen. A single specimen in DAOM on *L. michiganense* from Jefferson Co., Wisc. (H. C. Greene *1166*) bears cupulate aecia with spores 21–26 × 17–22.5 μ with hyaline walls, and is not associated with aecia or telia. It is probably a grass rust. An emended description, based on available North American material, follows:

Pycnia amphigenous or caulicolous. Aecia hypophyllous or caulicolous, surrounding the pycnia, on stems often extending for several inches, dull brownish yellow. Peridium weakly developed, not cupulate, the sori partly covered by the ruptured epidermis and so appearing bullate; peridial cells ca. 30–60 × 28–48 μ, shallowly verrucose on outer face, with slender cylindrical warts on inner face; aeciospores 22–36(–39) × 15–24.5(–26.5) μ; spore wall 0.8–1.8(–2.2) μ thick, subhyaline to light yellow, finely to moderately coarsely verrucose; pores barely visible, apparently ca. 5–7, scattered or in two bands. Uredinia amphigenous, golden brown, often scarce or suppressed; urediniospores 26–34(–36) × 21.5–30 μ; spore wall 1.5–3.2 μ thick, pale to medium yellow, irregularly echinulate at 1.0–3.5 μ spacing; pores (2–)3–5(–7), scattered or, especially when few, ± equatorial. Telia amphigenous, blackish brown; teliospores 26.5–47 × 20–29 μ; spore wall 1.5–3.2 μ thick, light chestnut, with shallow irregular longitudinal ridges at 1.8–3.5 μ spacing from center to center; pore with small semicircular, subhyaline papilla ca. 1.0–3.0 μ high and 3.0–4.5 μ wide; pedicel hyaline, deciduous.

Specimens examined: On *Lilium canadense* L., Ont.: New Durham, R. F. Cain (DAOM *4261, 14861*). On *L. columbianum* Hanson, B.C.: 15 miles NW of Port Alberni, Vancouver I. (C, P & T *16459*), 4 miles SSE of Nanaimo (C, P & T *16224*), 29 miles E of Quesnel (C, S & F *14160*), between Budge Lake and 70 Mile House, 4000 ft (C, P & T *18826*), Mt. Begbie near Clinton (C, P & T *19256*), near Horsefly (C, P & T *19015*), near Crawford Bay (C & S *9054*), SE of Little Shuswap L. (C & S *8670*), Goat R. Canyon near Erickson (C & S *9274*), 1 mile SW of Hope (C & S *8363*), between Hope and Kawkawa L. (C & S *10466*), near Nakusp, Upper Arrow L. (C & S *9976*); Wash.: Bellingham (N.A. Ured. *2190*); Calif.: Kings River Canyon (N.A. Ured. *1289*). On *L. michiganense* Farwell, Wisc.: Dane Co. (H. C. Greene *1225*). On *L. pardalinum* Kellogg, B.C.: Crawford Bay, H. Murray (DAOM *6755*); Calif.: 18 Mile Creek, Del Norte Co., J. P. Tracey (DAOM *4749*). On *L. parviflorum* (Hook.) Holtz., B.C.: Crawford Bay, J. W. Eastham (DAOM *6774*). On *L. philadelphicum* L., Que.: Mt. Orford, H. Groh (DAOM *4008*); Ill.: Lake Forest, R. A. Harper (DAOM *6435*). On *L. superbum* L., Ont.: London, J. Dearness (Can. F. *270*); Mich.: Ann Arbor (N. A. Ured. *889*). On *Lilium* hybrid, Ont.: Simcoe, J. W. Crow (DAOM *2125*). The rust on cultivated *L. pardalinum* and *L. parviflorum* at Crawford Bay, B.C., presumably spread from native *L. columbianum*, which is rusted in the area.

Of five available Japanese specimens, only two bear uredinia and telia. The rather limited numbers of spores seen suggest that the Japanese rust may have slightly smaller urediniospores than the North American rust, but the teliospores are in good agreement. The other three collections bear only aecia. The aecia are cupulate and the spores are 20–24(–27) × 16–23 μ, with hyaline to subhyaline walls and no visible pores. If these aecia belong to *U. holwayi* the Japanese rust is subspecifically distinct; but it is possible that they belong to some heteroecious species.

UROMYCES HETERODERMUS Syd., Ann. Mycol. **4**: 29. 1906.

In the following records the nomenclature of Applegate (**1935**) for *Erythronium* is followed, except that varieties and subspecies are ignored because they are seldom fully determinable in the rusted specimens. The principal change involved is that the plant long known as *Erythronium giganteum* Lindl. becomes *E. oregonum* Appleg., Lindley's type having proved to be *E. grandiflorum*.

On *Erythronium grandiflorum* Pursh, B.C.: Thetis L., near Victoria, Vancouver I. (C, P & T *16357*), Copper Mtn., near Nelson, 7000 ft (C

& S *10980*), Mt. Revelstoke Nat. Park, 6200 ft (C & S *10842*); Alta.: Quartz Hill, near Banff, I. M. Lamb (DAOM *33977*). On *E. oregonum* Appleg., B.C., Vancouver I.: 15 miles NW of Port Alberni (C, P & T *16460*), 6 miles SSE of Nanaimo (C, P & T *16193*), 3 miles E of Sooke (C, P & T *16287*), NW of Nanaimo (C, P & T *16481*), Victoria (M. C. Melburn *117*). On *E. revolutum* J. E. Sm., B.C.: Sidney, Vancouver I., J. Macoun (DAOM *582*). This rust was collected on *E. grandiflorum* by Holway at Glacier, Field and Prospector's Valley, B.C., but it is only in the last few years that we have obtained adequate distribution data. To judge from our experience with many other fungi, including several discussed in this paper, the records on *E. oregonum* are ascribable to favorable climatic conditions on the coast rather than to exceptional susceptibility of that species. It and *E. revolutum* should be examined for this rust farther south near the coast.

UROMYCES MIURAE Syd., Ann. Mycol. **11**: 94. 1913.

Recent field work in the Pacific northwest has shown this rust to be abundant and often heavy on *Fritillaria kamtschatcensis* along the coast, where conditions are favorable. It is much scarcer inland on *F. lanceolatum*, although that species seems to be fully susceptible. Recent records in DAOM include:

On *Fritillaria kamtschatcensis* (L.) Ker, Alaska: Naknek, Alaska Pen. (W. B. Schofield *2087*), Hope, Kenai Pen. (C *5205*); B. C. mainland: Galloway Rapids, near Prince Rupert (C, S & F *13172B*); B. C., Queen Charlotte Is.: Yakoun R. delta, Graham I. (C, S & T *21549*), McLinton Creek, Graham I. (C, S & T *21570*), Tlell, Graham I. (C, S & T *20862*), Moresby Camp, Moresby I. (C, S & T *21977*). On *F. lanceolata* Pursh, B. C.: Mara L., S of Sicamous (C, & S *8734*), 6 miles SE of Merritt (C, P & T *16826*).

The last three species reveal their relationship in the longitudinal rows of warts and the small hemispherical germ-pore papillae on the teliospores.

PUCCINIA CALOCHORTI Peck, Bot. Gaz. **6**: 228. 1881.

Although this rust was previously unrecorded from British Columbia, our first season's field work, in 1953, in the region showed it to be essentially coextensive with *Calochortus*. However, the aecia pass quickly and, on the wide-ranging *C. macrocarpus*, the telia are generally inconspicuous, often being confined to the leaf axils where dew presumably persists long enough to permit germination even in the arid Upper Sonoran

areas. Arthur (**1934**) records this rust on *C. macrocarpus* only from Oregon, but it is to be sought in Washington, Idaho and Montana on the basis of our experience.

British Columbia records include: On *Calochortus apiculatus* Baker, Goat R. Canyon, Erickson, near Creston (C & S *9275*), 1½ miles W of Waldo, E. Kootenay Valley (C & S *9203A*), 2 miles SE of Galloway, E. Kootenay Valley (C & S *9105*). On *C. macrocarpus* Dougl., 5 miles SW of Alkali Lake P.O., 51° 44′N 122° 19′W (C, P & T *17129*), 19 miles W of Spences Bridge (C & S *10396*), 12 miles W of Kamloops (C & S *10290*), 17 miles NE of Merritt (C, P & T *16811*), 3½ miles SE of Monte Creek between Vernon and Kamloops (C & S *10269*), between Vernon and Lumby (C & S *10952*), 2 miles NE of Naramata, near Penticton (C & S *10209*), W side of Richter Pass, 2 miles from U. S. border (C & S *9850*), W slope of Okanagan Valley, near Osoyoos, 1 mile from U. S. border (C & S *9874*), E of Osoyoos (C & S *9628*), foot of Mt. Anachist near Osoyoos (C & S *9837*), 8 miles S of Radium Hot Springs, E. Kootenay Valley (C & S *11200*), 1½ miles W of Waldo, E. Kootenay Valley (C & S *9203B*).

PUCCINIA MESOMAJALIS Berk. & Curt. ex Peck, Ann. Rep. N. Y. State Mus. **25**: 111. 1873.

Although this rust occurs on *Clintonia borealis* (Ait.) Raf. in eastern Canada from central Ontario to Newfoundland, nearly all our specimens are from the humid regions from Gaspé Pen. eastward. It is particularly abundant in Newfoundland and Gaspé Pen. In British Columbia it is apparently coextensive with *C. uniflora* (Schult.) Kunth, 17 specimens in DAO ranging southward from Pine Pass and 12 miles NW of Kispiox, in approximately the latitude of Hyder and Ketchikan, Alaska, from which it is reported by Cash (**1953**). We have one specimen from Crowsnest Forest Reserve, J. L. Van Camp (DAOM *1326*), which provides an Alberta record, lacking from Arthur (**1934**).

A feature of varying proportions of spores in the majority of collections, which does not appear in published descriptions, is that, superimposed upon the finely rugose wall pattern, there may be several delicate longitudinal ridges. Such ridges are plentiful in most collections from Newfoundland, but they also occur sporadically throughout the range and so are apparently of no phytogeographic significance. They are probably detectable only in heated lactophenol mounts, in which the spores are fully turgid and completely cleared. It is possible that they indicate a relationship to the series of *Allium* rusts to be discussed below.

Puccinia kukkonenis Savile, sp. nov.

Pycnia, aecia urediniaque ignota. Telia praesertim hypophylla. Teliosporae ellipsoideae, 27–39(–42) × 17.5–24.5 μ; paries 2.0–2.8 μ crass. verrucis inclusis, aurantio-brunneus, verrucis ca. 0.2–0.4 μ alt., 0.3–0.8 μ lat., 1.0–1.5 μ a centro ad centrum. Pori germinativi apicales et ¾–⅞ depressi, sine papillis. Pedicelli subhyalini deciduique.

On leaves of *Lloydia serotina* (L.) Reichenb., mountain 4 miles W of Hyland L., 5000–6000 ft, 62° 03′N 128° 59′W, Yukon (I. Kukkonen & J. A. Calder *581*, TYPE).

When Dr. Kukkonen brought this specimen back I tentatively determined it as *Puccinia bessei* D. Cruchet. Later, a part of the collection was sent to Dr. G. B. Cummins who sent, in return, a fragment of the type of *P. bessei* and a part of a collection of the newly rediscovered *P. pagana* Arth. The latter species, originally stated by the collector to have been on *Allium textile*, is quite unlike any *Allium* rust and is certainly on *Lloydia*. See Cummins and Baxter (**1962**) for discussion of this species. The Yukon rust is intermediate between the other two in morphology, and the distinctions seem to warrant it being treated as a full species. Should further *Lloydia* rusts eventually be found in other mountain systems that largely overlap the distinctions between these three rusts it may become preferable to treat them as subspecies; but, for now, I prefer to treat them as allopatric species. The Yukon rust unquestionably has spread from one of the Alaska, Yukon or western Mackenzie refugia, although the type comes from a glaciated area, but a dark brown rust on the linear leaves of *Lloydia* is inconspicuous, and it may be long before the range can be delimited.

For convenience, detailed descriptions of the other two rusts are included here:

Puccinia bessei D. Cruchet (Bull. Soc. Vaud. Sci. Nat. **44**: 469. 1909).

Teliospores (30–)32–43 × (18–)21–29.5 μ, ellipsoid or slightly constricted; wall 1.0–1.8 μ thick including warts, yellow-brown, minutely verrucose with warts up to ca. 0.2 μ high, ca. 0.2–0.3 μ wide, ca. 1.0 μ spacing from center to center; pores ± apical and ¾–⅞ depressed; pedicel hyaline and deciduous. On *Lloydia serotina* (L.) Reichenb., Hohlstein, Canton du Valois, Suisse, 20 July 1909, D. Cruchet (TYPE).

Puccinia pagana Arth. (Bull. Torr. Bot. Club **38**: 372. 1911).

Teliospores 29–40.5 × 22–27 μ, ellipsoid (to slightly constricted); wall 2.5–3.5 μ thick, including crowded and flat-topped warts; warts ca. 0.5–

1.3 μ high, 0.5–1.5 μ wide or occasionally to 2.3 μ long, 1.5–2.5 μ spacing from center to center; pores apical and ¾–⅞ depressed; pedicel hyaline or subhyaline, deciduous. On *Lloydia serotina* (L.) Reichenb., Brecchia Peak above Togwotee Pass, E of Moran, Wyo. (R. Peterson and G. B. Cummins *60–80*).

It is obvious that these three rusts are closely related, but it will also be noted that the wall thicknesses and the heights, widths and spacings of the warts show no appreciable overlap. Some mesospores occur in the type collection of *P. bessei* and some unrediniospores have been reported in the telia of *P. pagana;* but without a good series of specimens it is impossible to tell whether their occurrence has any taxonomic significance, for such occurrences are erratic in some rusts. It is doubtful whether the urediniospores of *P. pagana* indicate that it is a long-cycled species. High-arctic and high-alpine rusts are almost always short-cycled at least functionally.

PUCCINIA ASPARAGI DC., Fl. Fr. **2**: 595. 1805.

In his manual Arthur (**1934**) gives no detailed range for this relatively common rust. In North American Flora he (**1920**) gives only Manitoba and Ontario as the Canadian range. It may therefore be noted that there are specimens in DAOM from British Columbia, Saskatchewan, Manitoba, Ontario, and Nova Scotia. It is still seen occasionally despite the increased use of resistant varities. The species is mentioned here principally to point out that the description and illustration of the urediniospores in Arthur (**1934**) are erroneous, having evidently been taken from a mount of some other species. The spores are closely verrucose like those of *Uromyces limonii* (DC.) Lév., *U. inaequialtus* Lasch, *U. polygoni-avicularis* (Pers.) Karst., *Puccinia ellisiana* Thüm., *P. aristidae* Tracy, etc. The germ pores are generally 3–5, ± equatorial.

UROMYCES PRIMAVERILIS Speg., Anal. Soc. Ci. Argent. **12**: 72. 1881.

Examination of Spegazzini's Dec. Myc. Arg. 31, which, if not the type, is certainly authentic material of this species, suggested considerable distinction between it and the North American rust on *Nothoscordum*, originally described by Sydow as *Uromyces nothoscordi*, but reduced to synonymy with *U. primaverilis* by Arthur. The material of the latter in the Arthur herbarium was accordingly borrowed. Examination shows that the differences are less than anticipated but still appreciable. It is questionable whether they should be regarded as geographic subspecies or host-specialized varieties. However a single Mexican specimen is in

some respects intermediate in morphology and the former alternative is accordingly proposed. *Nothoscordum* is a predominantly South American genus, and it seems probable that some of its species may bear other rusts in this complex.

UROMYCES PRIMAVERILIS Speg. ssp. **primaverilis.**

Aeciospores 22–33 × 18–25 μ; wall 1.2–1.5 μ thick, hyaline, minutely verrucose; pores invisible; no pore plugs. Peridial cells 33–55 × 22–40(–45) μ, minutely verrucose on outer face, with slender warts to ca. 6 μ long on inner face. Teliospores 26–37 × 19–30 μ; wall 2.0–4.0(–4.5) μ thick, deep yellow-brown, with fine longitudinal ridges at 1.3–1.6 μ spacing; germ pore apical to occasionally ½ depressed, with hyaline rounded papilla 1.0–2.0 μ high, 3.0–4.5 μ wide; pedicel hyaline, deciduous close to spore.

On *Allium striatellum* Lindl., Parque de Palerme, Argentina (Speg. Dec. Myc. Arg. *31* in DAOM).

UROMYCES PRIMAVERILIS Speg. ssp. **nothoscordi** (Syd.) Savile, comb. nov.

Uromyces nothoscordi Syd., Hedwigia **40**: 125. 1901.

Aeciospores 21–29 × 16–23.5 μ; wall 0.6–1.5 μ, minutely verrucose; pores invisible; no pore plugs. Peridial cells 27–41(–46) × 16–28(–31) μ, minutely verrucose on outer face, with slender warts ca. 3–7.5 μ long on inner face. Teliospores 21–32(–36) × 16–23(–25) μ; wall 1.3–2.8 μ thick, yellow-brown, with fine longitudinal ridges at 1.2–1.8 μ spacing; germ pore apical to occasionally ½ depressed; pedicel hyaline, deciduous near spore.

On *Nothoscordum bivalve* (L.) Britt. Texas: Austin, W. H. Long (PUR *34913*, TYPE), San Antonio, Epeling *160* (PUR *34911*) and Griffiths (PUR *34907*); Ill.: Cobden, A. B. Seymour *4108* (PUR *34908*). On *N.* ? *texanum* M. E. Jones (as *N. "arizonicum,"* perhaps a *lapsus calami*, *N. texanum* having been described from Arizona): Jones Recreational Area, Hwy. 60, Ariz., L. N. Goodding *41–41* (PUR *50564*).

There is one collection in the Arthur Herbarium, collected as "on *Allium*," perhaps on *Nothoscordum bivalve* but very fragmentary, that does not agree with the above specimens. This is from Toluca, south of Mexico City, Mexico, Holway *3132* (PUR *34912*), a substantial distance from any other specimen. The aeciospores are 25–32 × 19–25 μ; wall 0.6–1.0 μ thick, minutely verrucose. Peridial cells are 28–47(–59) × 22–32 μ. Teliospores are 21–30 × 16–24 μ; wall 1.3–3.0 μ thick, with

very fine, scarcely visible longitudinal ridges; and the pores often lateral. This seems to be a distinct subspecies, but until more abundant material with fully determinable host specimens is available I prefer not to name it. More abundant specimens, accompanied by intact host plants, are needed for this whole complex.

PUCCINIA GRANULISPORA Ell. & Gall. ex Ell. & Ev., Bull. Torr. Bot. Club **22**: 61. 1895.

This is by far the commonest rust of *Allium* in western Canada, where it is nearly coextensive with *A. cernuum* Roth, a plant of great altitudinal diversity. Twenty-six Canadian specimens on this host in DAOM range from Fort St. John, B.C., and Peace River, Alta., in the north, to Vancouver I. in the southwest and Banff, Alta., in the southeast. Mature aeciospores are generally (24–)26–36 × 20–28(–31) μ, with wall 0.8–1.8 μ thick. Arthur's (**1934**) measurements evidently included extremely immature spores. Urediniospores are 28–36(–42) × 25–32(–34) μ; wall 1.3–2.0 μ thick, light yellow-brown, finely echinulate at 1.2–2.2 μ spacing; pores scattered, (8–)10–15(–17), generally distinct. Teliospores are (33–)40–73(–77) × 22–34(–38) μ, usually slightly constricted; wall 0.8–2.0 μ below, (2.2–)3.0–7.0 μ at apex, yellow-brown to chestnut, smooth except for a few delicate longitudinal ridges on many spores (visible only in thoroughly cleared spores); no evident germ pores; pedicel pale yellow, thin-walled but not truly deciduous; mesospores occasional; fused yellow-brown paraphyses sparingly produced, and telia pulvinate rather than firm. Colorado and Wyoming should be added to the range of this rust on *A. cernuum* (see discussion under *P. blasdalei*); and *A. recurvatum* should be deleted, it being synonymous with *A. cernuum*.

PUCCINIA BLASDALEI Diet. & Holw. in Diet., Erythea **1**: 248. 1893.

Recent records, culled in large part from phanerogamic specimens in DAO, give some host and range extensions. On *Allium acuminatum* Hook., B.C.: Victoria (M. G. Dudly *239*), Shawnigan L., near Cowichan, Vancouver I. (C, P & T *16262B*), Yale, lower Fraser Canyon, (P. P. Henson). On *A. amplectens* Torr., B.C.: 5 miles WSW of Sooke, Vancouver I. (C, P & T *16269*); Calif.: Schoolhouse Hill, Siskiyou Co. (R. S. Ferris and L. Lorraine *11720*), 1 mile N of Orland, Glenn Co. (L. S. Rose *50048*). On *A. punctum* Henderson, Ore.: 16 miles N of Scotts Butte, Malheur Co. (C. L. Hitchcock & C. V. Muhlick *21101*). On *A. lemmonii* S. Wats. (syn. *S. scissum*), Ore.: 16 miles N of Scotts Butte, Malheur Co. (C. L. Hitchcock & C. V. Muhlick *21100*). From

the last two specimens, in colonies that were almost contiguous (C. L. Hitchcock, in lit.), it appears likely that a single strain attacks these two species. On *A. deserticola* (M. E. Jones) Woot. & Standl., N. Mex.: Tortugas Mtn., Dona Ana Co., I. H. Crowell (DAOM *5063*), host too fragmentary for confirmation.

These collections agree well with the description of Arthur (1934) except that the teliospores are seldom more than 60 μ long. It seemed probable that Arthur's high maximum length was due to his including some specimens of *P. granulispora* on *A. cernuum*. Study of the *A. cernuum* rusts in PUR assigned to *P. blasdalei* shows that all are typical *P. granulispora*. Thus *A. cernuum* should be deleted from the hosts of *P. blasdalei*, and Colorado and Wyoming deleted from the range. It is not clear how this error arose, since several early specimens are annotated by E. B. Mains as having very slight stroma (i.e. fused paraphyses). Arthur's record of this rust on *A. textile* is based on an aecial specimen of *P. mutabilis*. Thus *P. blasdalei* occurs only west of the Rockies. It is distinguished from *P. granulispora* by its slightly smaller aeciospores (23–31 × 16–25 μ); smaller urediniospores (23–33 × 20–26 μ) with 8–14 pores; smaller teliospores (33–64 × 17–28 μ), with wall 3–8 μ thick at apex and pedicel yellow and firm; mesospores scarce to moderately common; and brown, fused paraphyses abundant, dividing the very firm telia into small locules fused to the epidermis. The teliospores occasionally bear a few faint longitudinal ridges.

PUCCINIA MIXTA Fckl., Symb. Myc., 58. 1870.

This seems to be the preferred name for the introduced rust on *Allium schoenoprasum* L., which Arthur called *P. porri*, and which some authors, lumping all the European rusts of *Allium* together, call *P. allii*. However, the distinctions seem to be ample to merit specific rank for each of these rusts. Canadian specimens in DAOM, all from southwestern British Coumbia, are: On *Allium cepa* L., Victoria, W. R. Foster (DAOM *6858*). On *A. schoenoprasum* L., Ten Mile Point, Victoria, H. T. Güssow (DAOM *19795, 23398*), Vancouver, J. W. Eastham (DAOM *14082*). The late Dr. Güssow noted that the rust killed the chives leaves every year but that new growth came up and was sound for about a year. Probably the rust flourishes in the spring but is generally checked by dry summer weather. The rust seems to persist readily in plantings of chives in home gardens, whence it may spread occasionally to onion. In these specimens the urediniospores are 24–33 × 21–26.5 μ, with wall 1.0–1.7 μ thick, light yellow-brown, finely echinulate at 1.3–

2.0 μ spacing and pores 8–11, scattered. Two-celled teliospores are 32–43 × 19–25(–27) μ, with wall 1.0–1.7 μ thick below and 1.8–3.3 μ above, light chestnut, smooth except generally a few fine longitudinal or anastomosing ridges, generally no visible germ pores, pedicel ± hyaline and freely deciduous; one-celled spores fully as common as two-celled ones, 26–36 × 16–23 μ, with anastomosis of the delicate ridges often extensive. Although Fuckel's description, based on a rust on this host, from his garden, is not adequate by modern standards, he noted that the teliospores are sometimes septate, sometimes simple, and his illustration shows the very slight apical thickening and the hyaline deciduous pedicels. Our specimens are a perfect match for specimens from Sweden and Denmark.

Puccinia porri Wint. is the rust of *Allium porrum* L., which has apparently never reached North America. Only uredinial material is available in DAOM, but these specimens (Roum. F. Sel. *2344* and IMI *24013*) show that this rust is clearly distinguished by its urediniospores. The spores are 27–36(–38) × 23.5–30 μ; the wall is 1.5–3.0 μ, pale yellow-brown, with conspicuous echinulations at 1.5–2.5 μ spacing; and the pores (?8–10, scattered) are scarcely visible in face view, but are conspicuous in equatorial view because of the marked local thickening of the wall and a pronounced hyaline "blister" over each one. In the remaining rusts of *Allium* discussed in this paper there is little wall thickening round the pores, and almost no blister-like cap; and the echinulations are so delicate as to be almost invisible in equatorial view in lactophenol mounts, whereas they are plainly visible in this species. *P. porri* thus is distinguished by urediniospore size, wall thickness, pore morphology, echinulation size and echinulation spacing.

Hylander, Jørstad and Nannfeldt (**1953**) made *Allium oleraceum* L. the lectotype host for *Puccinia allii* Rud. (Linnaea **4**: 392. 1829). In the only specimen available on this host (Syd. Ured. *2727*) the telia resemble those of *P. blasdalei* in that numerous fused paraphyses produce a firm, locular sorus; but the teliospores are somewhat smaller (35–55 × 17–25 μ), the wall is 2.0–5.0 μ thick at the apex, and the pedicel is hyaline and deciduous; no pores are discernible.

PUCCINIA MUTABILIS Ell. & Gall., Jour. Mycol. **5**: 67. 1889.

This species is reported by Arthur (**1934**) north to South Dakota in the eastern Cordillera, but we have three specimens from Alberta, and there is one in PUR. On *Allium geyeri* S. Wats.: Stettler, Camrose Dist. (A. H. Brinkman *2795*), and Waterton River Crossing, Waterton

Lakes Nat. Park (A. J. Breitung *16822*). On *A. textile* Nels. & Macbr.: Kinsella, 52°30'N 111°20'W (E. H. Moss *2571*), and Craigmyle Dist., Brinkman *1747* (PUR *30623*), aecia only, the source of Arthur's record of *P. blasdalei* on this host. In view of its occurrence in Waterton Park this rust is to be expected in the adjacent Glacier Park, Mont. The aeciospores are 17–22 × 16–20 μ, with wall ca. 0.7–1.0 μ thick. Arthur gives the urediniospore pores as 4–8, but in these specimens, although they are sometimes obscure, they appear to be 8–11. The teliospores are broadly rounded above, 32–44 × (20–)22–30(–33) μ; the walls are 1.3–2.5 μ thick at the sides, 1.5–3.0 μ above, orange-brown to light chestnut, smooth; no pores visible; pedicels pale yellow and raggedly deciduous; mesospores scarce and paraphyses lacking.

UROMYCES AEMULUS Arth., Bull. Torr. Bot. Club **28**: 373. 1911.

Although closely related to *Puccinia mutabilis,* this species occurs predominantly west of the Rockies and has not been reported on *A. geyeri* and *A. textile,* the principal hosts of that rust. It is probably a quite recent segregate. The following host and range extensions may be noted. On *Allium acuminatum* Hook., B. C.: Shawnigan L., near Cowichan, Vancouver I. (C, P & T *16262*A); Wash.: 5 miles S of Trout L., Klickitat Co. (F. G. Meyer *1518*). On *A. cusickii* S. Wats., Ida.: near Lewiston, Nes Perces Co. (A. A. & E. G. Heller *3054*). On *A. douglasii* Hook., Ore.: 12 miles SE of Freewater, Umatilla Co. (C. L. Hitchcock & C. V. Muhlick *20980*).

In these specimens the urediniospores are 27–34 × 23–28 μ, with ca. 10–16 pores (more than in *P. mutabilis*). The teliospores are 24–34(–36) × 19–25.5 μ, the wall is 1.5–3.5 μ at the apex, no pore is visible, and the pedicel is yellowish, delicate and irregularly deciduous.

I have discussed the morphology of these *Allium* rusts in considerable detail not only to emphasize their interrelationships and specific distinctions, but also because some morphological characters strongly indicate that they are related to, although more primitive than, the *Uromyces muscari* complex discussed below. The characters that indicate such a relationship are the almost invariable lack of a teliospore germ pore, the teliospore wall pattern of widely separated longitudinal to anastomosing ridges, and several scattered pores in the urediniospores. The first two of these characters are so scarce as to command attention even if they occurred singly. Taken together they are even more impressive. This link between the parasites of *Allium* and of Liliaceae *s. str.* makes one hesitate to accept Hutchinson's (**1934**) transfer of *Allieae* to Iridaceae, a course since followed by a few other authors.

UROMYCES MUSCARI (Duby) Lév., Ann. Sci. Nat. Bot. III, **8**: 376. 1847.

Uredo muscari Duby, Bot. Gall. **2**: 898. 1830.

Uromyces scillarum (Grev. ex Berk.) Lév. Ann. Sci. Nat. Bot. III, **8**: 376. 1847.

Uredo scillarum Grev. ex Berk., J. E. Sm. Engl. Fl. **5**: 376. 1836.

Uromyces scillarum (Grev. ex Berk.) Wint., in Rabh. Krypt. Fl. **1**: 142. 1881.

Puccinia scillarum Baxt., Stirp. Crypt. Oxon. 40. 1825.

Uromyces concentricus (Desm.) Lév., Ann. Sci. Nat. III, **8**: 371. 1847.

Uredo concentrica Desm., Ann. Sci. Nat. Bot. III, **6**: 62. 1846.

The above partial synonymy is given because great confusion has surrounded the nomenclature of this species. The confusion stems largely from the erroneous belief, possibly originating with Saccardo, that volume 5 of Smith's English Flora was issued in 1826 rather than 1836. This error has been copied repeatedly. Jørstad and Nannfeldt (**1958**) seem to be the first authors to realize that *Uromyces muscari* is the valid name for the collective species.

The nomenclature was further greatly complicated by Schneider (**1927**). Schneider, using a single strain of rust from *Muscari racemosum*, obtained infection on this host and on one strain of *M. neglectum*, but not on another strain of *M. neglectum* or on *M. botryoides*, *M. comosum*, *Scilla bifolia*, *S. maritima* and *S. nonscripta*. It was thus clear that some host-specialization occurred; but no other hosts were inoculated and no use was made of inoculum from other hosts; and the degree of specialization was therefore in doubt. Schneider also learned how to germinate the teliospores and showed that germination occurs through a split in the spore wall, there being no germ pore. Schneider made some morphological comparisons of the rusts from several hosts and split the species into seven subspecies on the basis of apparent spore size differences and presumptive host specialization. He generally examined only one specimen on any host, his dimensions are often so unrealistic that one must conclude that many of the spores in his mounts were collapsed, and he ignored spore wall characters. After examining material on several of the same hosts, occasionally from the same locality or even the same collection, I find no basis for most of Schneider's supposed size differences. Schneider concluded from his comparison that *Uromyces scillarum* was a collective species and stated (p. 259) that he

was splitting it into subspecies. He then proceeded to list seven entities written in binomial form, i.e. as species, two of which he stated to be new subspecies. Whether this is a legitimate way of making subspecies is fortunately an academic problem, for *U. muscari* is included in the collective species, and *muscari* is the valid specific epithet. The more difficult problem is to know what to do with the names that he wrote in specific form. Some authors have credited him with some of these "species." Thus he wrote "*Uromyces hyacinthi*" and may, perhaps, be credited with erecting a new species. This name has been written as *U. hyacinthi* (Opiz) Schneider, but there is nothing to indicate that he was making a recombination of *Uredo hyacinthi* Opiz (in Sched. et Seznam, 152. 1852). His most remarkable action was, in looking for a basionym for his proposed subspecies on *Muscari racemosum*, to write "*Uromyces limbatus* Rabh." This name proves to have been based (as his literature reference shows) on *Uredo limbata* var. c. *muscari* Rabenh. (Krypt. Fl. I: 12. 1844). It seems wisest to judge all his combinations to be not validly published as species on the basis of the following statement in Article 32 of the International Code: "A combination is not validly published unless the author definitely indicates that the epithet or epithets concerned are to be used in that particular combination." In these examples there is no such indication; the author writes one thing and says he intends another.

Recently Lehoczky (**1959**) has made reciprocal inoculations and a morphological study of *Uromyces muscari* on several hosts. He concluded that there was no morphological distinction in the rusts that he studied, with which I am in full agreement (see below). Because of definite host specialization he set up four formae speciales of *Uromyces scillarum:* f. sp. *hyacinthi*, f. sp. *muscari-comosi*, f. sp. *muscari-racemosi* and f. sp. *scillae*. This seems to be a logical disposition of the complex; but, to be valid, these taxa must be based on *Uromyces muscari;* and, to conform with Article 26 of the Rules, f. sp. *muscari-comosi*, the type form, must be written f. sp. *muscari*. To validate these names the formal transfers are given herewith. *Uromyces muscari* (Duby) Lév. f. sp. *muscari* on *Muscari comosum; U. muscari* f. sp. **muscari-racemosi** (Lehoczky) Savile, comb. nov., on *Muscari racemosum; U. muscari* f. sp. **hyacinthi** (Lehoczky) Savile, comb. nov., on *Hyacinthus orientalis; U. muscari* f. sp. **scillae** (Lehoczky) Savile, comb. nov., on *Scilla bifolia.*

Uromyces muscari occurs naturally from western Europe to central Asia; but it has been sparingly introduced into other regions, including California, British Columbia and New Zealand. It was reported by Arthur (**1934**) from California on *Scilla hispanica* Mill., but according

to Dr. G. B. Cummins (in lit.) has not been reported again in the United States. From a specimen that reached this institution only in 1955, it is known that this rust occurred on *Hyacinthus orientalis* L., in 1919, in a garden at Sidney, Vancouver I., B. C. (DAOM *49814*). In the spring of 1961 Mr. H. N. W. Toms sent in abundant material of the rust from Victoria, B. C., on *Scilla hispanica* Mill. (DAOM *75626*). Mr. Toms states that he first saw the rust in this garden a few years previously but that heavy rain discouraged him from making a specimen. On his return visit in 1961 the rust was abundant in the same part of this old, partly wild garden, but had not spread noticeably. It thus seems that the rust may persist indefinitely under the conditions of southeastern Vancouver I., but that it is unlikely to be a significant pest.

The following description is based on the two British Columbia specimens, a specimen on *Scilla* sp. from Christchurch, New Zealand, J. M. Dingley (Herb. Pl. Dis. Div. N. Z. *19093*), and 14 European specimens on *Hyacinthus ciliolatus* Poir., *Muscari comosum* Mill., *M. neglectum* Guss., *M. racemosum* (L.) Mill., *Scilla hispanica* Mill., *S. maritima* L., and *S. nonscripta* (L.) Hoffm. & Lk. Included is Baxt. Stirp. Oxon. *40*, the type of *Puccinia scillarum* Baxt. This specimen is of interest because Baxter's name is the first applied to the species. It cannot be used, however, because the transfer to *Uromyces* would make a later homonym. In all these specimens I find no safe distinction for the rust on any one host. The variation between specimens on one host, and often even between sori of one specimen, is about as great as between specimens on different hosts.The following description is based on warmed lactophenol mounts of turgid spores, with two or more sori represented in each mount.

Teliospores 19–34 × 14–25 μ, globoid, ellipsoid, clavate or irregularly angular; wall 1.2–3.0(–3.5) μ thick, very slightly more at apex than at sides, orange-brown or light chestnut, smooth or rarely with a few small scattered warts, often with a few shallow distant longitudinal or anastomosing ridges (seldom at less than 5 μ spacing); germ pore apparently always lacking; pedicel light yellow, irregularly deciduous (i.e. lacerate rather than abscissing cleanly near spore).

To indicate the variability on a given host the range of dimensions for the 17 individual specimens examined is given, with the variation for three specimens on *M. comosum* following in parentheses: Min. length, 19–23(20–23) μ; max. length, 29–34(31–33) μ; min. width, 14–18(14.5–16.5) μ; max. width, 22–25(23–25) μ; min. wall thickness, 1.2–1.8(1.3–1.5) μ; max. wall thickness, 2.0–3.5(2.2–3.5) μ. It is thus clear that there is little or no morphological differentiation between hosts.

A related, but definitely distinguishable species, *U. circinalis* Kalchbr. & Cke. (Grevillea **8**: 71. 1879, syn. *U. macowani* Bubák), occurs in South Africa on *Scilla prasina*. Thuem. Myc. Univ. *1438* yields teliospores 16.5–28(–31) × (14.5–)15.5–25(–28) μ, globoid to occasionally ellipsoid or slightly angular; wall (1.5–)2.0–4.0(–4.5) μ thick, yellow-brown, smooth but often uneven although lacking definite ridges; no germ pores seen; pedicel ± hyaline, deciduous near spore.

A species that is more questionably distinct from *U. muscari* is *U. algeriensis* Syd. (Monogr. Ured. **2**: 281. 1910), which occurs in the Mediterranean region. It is distinguished in part by the regular presence of urediniospores in the telia. Urediniospores have been reported for *U. muscari*, but none are present in any of the specimens examined by me. One specimen in DAOM is assignable to *U. algeriensis*, on *Bellevalia flexuosa* Boiss., Dir-Amar, near Jerusalem, Israel, T. Rayss (DAOM *46215*), issued as *U. scillarum*. The urediniospores are 23–33 × 21–22 μ, with wall ca. 1.5 μ thick, subhyaline, minutely echinulate at ca. 1.0–2.9 μ spacing, pores ± obscure, several, scattered. Teliospores are 19–29.5(–33) × 14–23.5 μ, globoid, ellipsoid or irregular, but not sharply angled; wall 1.3–2.2 μ thick, orange-brown, smooth or often with fine ± longitudinal but occasionally anastomosing ridges sometimes at less than 2.0 μ spacing (closer than in *U. muscari*); no visible germ pores; pedicel yellowish, deciduous.

FUNGI IMPERFECTI

CYLINDROSPORIUM SMILACINAE Ell. & Ev., Bull. Torr. Bot. Club **27**: 58. 1900.

Found on *Smilacina racemosa* (L.) Desf., 2 miles W of Taghum, near Nelson, B. C. (C & S *9361*), ca. 30 miles N of the Washington boundary. Spores 46–111 × 2.2–3.0 μ, hyaline, with ca. 2–4 septa. Spores and lesions agree well with those in Rel. Suks. 13. Shaw (**1958**) reports it from Washington, Oregon and Idaho (type locality); but if, as seems probable, *Phleospora vagnerae* Petrak (Ann. Mycol. **20**: 209. 1922) is a synonym, Montana should be added. There are two Quebec specimens on the same host in DAOM that probably belong here, but which have spots with little necrotic tissue and abundant anthocyanin. One, from Kingsmere, Gatineau Co., I. L. Conners (DAOM *3405*) has spores that agree with the western material except that there are up to ten septa. The second, from Rougemont, Rouville Co., L. Cinq-Mars (DAOM *41156*) has much shorter, continuous spores and seems to be very immature.

PHYLLOSTICTA MELANOPLACA Thüm., Bull. Soc. Nat. Mosc. **55**: 230. 1880.

On *Veratrum eschscholtzii* A. Gray, 4 miles south of Juskatla, Graham I., Queen Charlotte Is., B. C. (S *3545*). Abundant, forming elongate black lesions on the leaves, which bear pycnidia intermixed with perithecial initials. Spores bacillar to slightly dumbell-shaped, 3.0–4.8 × 0.6–0.9 μ. This is clearly the microconidial state of a pyrenomycete. It should be sought on the Pacific coast and material taken for overwintering. Reported by Weiss and O'Brien (**1952**) from Calif., Colo., Ida., Utah and Conn., and by Cash (**1953**) from Ketchikan, Alaska, about 120 miles north of the specimen reported here.

SEPTORIA STREPTOPODIS Peck in M. E. Jones, Univ. Mont. Bull. Biol. Ser. **15**: 64. 1910.

In specimens on *Disporum majus* (Hook.) Britt. from Vernon, B. C., J. Boyce (DAOM *1301*), *D.* sp., Campbell River, Vancouver I., B. C., W. G. Ziiler (DAVFP *8346*), and on *D.* sp. from Ione, Wash., A. W. McCallum (DAOM *1502*), the spores are 31–72 × 3.0–3.5 μ and 3–4-septate, only slightly narrower than the dimensions given by Peck for the fungus from Montana on *Streptopus roseus* (probably var. *curvipes*) and *Prosartes trachycarpa* (= *Disporum trachycarpum*). A specimen on *D. hookeri* (Torr.) Britt. var. *oreganum* (S. Wats.) Q. Jones, from N of Clearwater Sta., North Thompson R., B. C. (C, P & T *19914*) yielded spores 47–66 × 2.0–2.7 μ with 1–3 septa. These are probably all the same fungus. We have one Ontario specimen that is placed here with considerable misgivings, on *Streptopus roseus* Michx., Raleigh Falls, Kenora Dist. (P *413*), with spores 37–79 × 1.4–1.7 μ, 0–1-septate.

CERCOSPORELLA INCONSPICUA (Wint.) Höhn., Ann. Mycol. **1**: 413. 1903.

Cylindrosporium inconspicuum Wint. in Rabenh., F. Europ. *3178*. 1884. (Also 34. Jahresb. Nat. Ges. Graubündens, 69. 1890).

Cercosporella hungarica Bauml., Verh. Zool. Bot. Ges. Wien **38**: 716. 1888.

Cercosporella liliicola (Rich.) Sacc., Syll. **10**: 566. 1892.

Cercospora liliicola Rich., Cat. Champ. Marne, no. 2032. 1889.

Cercosporella lilii Dearn., Mycologia **21**: 327. 1929.

As was pointed out by Höhnel, Rabenh. F. Europ. *3178* bore a diagnosis of Winter's fungus, which gives his epithet clear priority. There

can be little doubt that all these fungi are conspecific. Dearness inadvertently omitted spore dimensions from the description of *C. lilii*, but he wrote 35–75 × 4–5 μ on the type packet. Examination of the type, on *Lilium canadense* L., N of Hudson Falls, N. Y., yielded conidia (32–)49–111 × (3.0–)3.6–4.8 μ with (1–)3–7(–8) septa. There are two Canadian records of *C. inconspicua*: On *L. martagon* L. × *L. hansoni* Leicht., Dropmore, Man., W. A. F. Hagborg & W. E. Sachston (DAOM *37914*), conidia (32–)49–100(–139) × 2.7–5.2 μ, with 2–7 septa. On *L. philadelphicum* L. var. *andinum* (Nutt.) Ker, 3 miles S of Lac la Biche, Alta. (W. J. Cody & R. L. Gutteridge *7132*), conidia 43–94 × 3.0–4.5 μ, with septa generally obscure but sometimes at least 4–5. These three specimens embrace the ranges of the various published descriptions and of two available European specimens on *L. martagon* (D. Sacc. Myc. Ital. *786;* and Hersbruck, Germany, K. Starcs, DAOM *20134*).

CERCOSPORELLA VERATRI Peck, Ann. Rep. N. Y. State Mus. **44**: 27. 1891.

A trace occurred, with *Puccinia veratri*, on *Veratrum eschscholtzii* A. Gray, on Mt. Thornhill, near Terrace, B. C. (C, S & F *14863B*); conidia 57–86(–94) × 2.2–3.2 μ, with 3–8(–10) septa, hyaline, with extremely slender flagellate tip ca. half length of spore; conidiophores generally less than 20 μ long. The isotype in Herb. Dearness agrees except that the spores run to 74 μ long and to 4.0 μ wide, with 3–4 septa. I find no other report of this species since Peck described it. It may be noted that *C. terminalis* Peck is *Cylindrosporium veratrinum* Sacc. & Wint.

RAMULARIA SMILACINAE J. J. Davis, Trans. Wis. Acad. Sci. **15**: 777. 1907.

Found on *Smilacina racemosa* (L.) Desf., B. C.: near Fort St. James (C, S & F *13835*), and W slope of Monashee Pass (C & S *9964*). This organism is reported by Shaw (**1958**) from all the states of the Pacific northwest but not from B. C. The Fort St. James station (54°20′N) is a substantial northward extension.

RAMULARIA SUBSANGUINEA (Ell. & Ev.) Savile, Can. J. Bot. **35**: 205. 1957.

This species is very common, although generally in poor fruit, on *Maianthemum dilatatum* (Howell) Nels. & Macbr. in the Queen Charlotte Is., B. C., and to a lesser extent on the coastal mainland. The fol-

lowing records are additional to those previously reported (Savile, 1957): Mainland: Aldergrove, lower Fraser Valley (C, S & T *20826*); Queen Charlotte Is.: Langara I. (C, S & T *22560*), near Massett (C, S & T *21239, 21286*), Queen Charlotte City (C, S & T *23026*), Sandspit (C, S & T *21094*). It is reported plentifully on this coastal species from southern Alaska to Oregon, but it is scarce inland. We have a single record on *M. canadense* Desf. var. *interius* Fern., 22 miles N of Fort MacLeod (C, S & F *13962*), in the northern Rocky Mountain trench and more than 300 miles inland.

SUMMARY

Puccinia kukkonenis is described on *Lloydia serotina* from southeastern Yukon. The following recombinations are made: *Uromyces primaverilis* Speg. ssp. *nothoscordi* (Syd.) Savile; *Uromyces muscari* (Duby) Lév. f. sp. *muscari-racemosi* (Lehoczky) Savile, f. sp. *hyacinthi* (Lehoczky) Savile, and f. sp. *scillae* (Lehoczky) Savile. *U. muscari* is shown to be allied to a complex of *Allium* rusts. Many host and range extensions are listed for various other fungi, notably *Ustilago heufleri* and *Cercosporella veratri*, neither previously reported from western North America.

ACKNOWLEDGMENTS

I am indebted to Dr. G. B. Cummins and Dr. J. W. Baxter for information and the loan of specimens, to the Director, Fielding Herbarium for the type of *Puccinia scillarum*, to Dr. C. L. Hitchcock for information, and to Dr. Marion Ownbey for checking *Allium* specimens. As is clear from the specimen citations, many of the records presented stem from the vigorous field program in the northern Cordillera conducted by Mr. J. A. Calder of this Institute.

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INFECTION WITH HISTOPLASMA DUBOISII IN DIFFERENT EXPERIMENTAL ANIMALS

MASAHIKO OKUDAIRA AND JAN SCHWARZ [1]

(WITH 5 FIGURES)

Large forms of *Histoplasma* from Africa have been reported as early as 1947 (**9**). *Histoplasma duboisii* was first found and named by Vanbreuseghem et al. in a patient who had returned from the Belgian Congo (**8**). The description of *H. duboisii* as a new species was based on the morphology of this fungus in the parasitic phase; it had large (13–15 × 10 μ) budding, ovoid cells, with a thick cell membrane (**20, 21, 22, 23**).

In early papers we concluded that from cultural findings alone, the African strains do not need to be considered as separate species (**6, 7, 18**). But later we found it possible to differentiate *H. duboisii* from *H. capsulatum* by its size in the parasitic phase, and by its different pathogenicity and particular tissue response in hamsters and in mice (**24**).

From a review of cases of African histoplasmosis, Quenum-Ahina in 1958 (**15**) concluded that *H. duboisii* was only a variant of *H. capsulatum*.

It is the purpose of this paper to report some morphologic features of *H. duboisii* in tissue.

MATERIALS AND METHODS

Six guinea pigs, 7 rats, 5 hamsters, 5 rabbits and 4 pigeons were used in this experiment.

H. duboisii (strain 44)[2] grown for 48 hours on blood agar media at 37° C was suspended in physiologic saline solution (10×10^6 cells ml). All animals were inoculated intraperitoneally with 0.5 ml of the sus-

[1] From the Clinical Laboratories, Jewish Hospital, Cincinnati and the Laboratory of Mycology (Departments of Dermatology and Pathology), College of Medicine, University of Cincinnati. This study was supported in part by grant E-576 of the National Institutes of Health.

[2] Strain "44" was isolated from a localized "African histoplasmosis" and obtained from Dr. E. Drouhet from the Institut Pasteur in Paris (**6**).

TABLE I

SUMMARY OF CULTURAL, HISTOPATHOLOGICAL AND MORPHOLOGICAL FINDINGS OF THE TISSUE PHASE OF HISTOPLASMA DUBOISII IN DIFFERENT EXPERIMENTAL ANIMALS

Animal	Days after inoculation	Culture**	Omentum					Liver					Spleen					Lung				
			G	A	S	M	L	G	A	S	M	L	G	A	S	M	L	G	A	S	M	L
Guinea pigs	3*		+	++	+⊥	⊥	−	−	+	+	−	−	−	+	+	−	−	−	+	+	−	−
	7		+++	++	⊥	+	⊥	+	+	⊥	−	−	−	+⊥	⊥	+	−	−	−	−	−	−
	14	+	++⊥	++	⊥	+	⊥	++	+⊥	⊥	⊥	⊥	−	+⊥	⊥	⊥	⊥	+	−	−	−	−
	21	−	++	+++	+	+⊥	⊥	+	+	⊥	⊥	−	−	+	+	−	−	++	−	−	−	−
	28	−	+⊥	++⊥	⊥	+⊥	⊥	+⊥	+	+	−	−	−	+	+	−	−	++	+	+	−	−
	35	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−
Rats	7	−	++++	++++	++	+⊥	⊥	++	+	+	−	−	++	+	⊥	⊥	−	++	+	⊥	⊥	−
	14	−	+++	++++	++	+	+	++	+	+	−	−	++	+	⊥	⊥	−	++	+	+	⊥	−
	21	+	+++⊥	++++	++	+	+	+⊥	++	⊥	+⊥	−	++⊥	++⊥	⊥	+⊥	⊥	+	+	⊥	⊥	−
	28	−	+	+	−	+	−	+	+	−	+	−	+	++	⊥	+	⊥	−	+	−	−	−
	35	−	++++	+++	++	⊥	⊥	+					+	++⊥	−	+	⊥	+	+	+	⊥	⊥
	49	−	+	++	+⊥	⊥	−	+	−	−	−	−	+⊥	++	⊥	+	⊥	−	−	−	−	−
	74	−	+++	++	+	⊥	⊥	+	+	−	+	−	+⊥	++	⊥	+	⊥	+⊥	−	−	−	−
Hamsters	21*		++++	+++⊥	++	+	⊥	++	+⊥	+	⊥	−	++	+⊥	⊥	⊥	⊥	+	+	⊥	⊥	−
	95*		++++	+++	++	⊥	⊥	+++	++⊥	⊥	+	−	+++	+++	⊥	+	+⊥	+	+	−	+	−
	134		++++	++++	⊥	+⊥	++	+++	++⊥	⊥	⊥	+⊥	+++	+++⊥	⊥	+	++	++	++⊥	−	+	+⊥
	164		+++	+++⊥	+⊥	+	−	+++	++	++	−	−	+++	+++	+	+	−	+++	+	−	⊥	−
	167		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	⊥	−
Rabbits	7	+	−	−	−	−	−	++	++	+⊥	⊥	−	+⊥	+	+	−	−	++	+⊥	+	⊥	−
	14	−	−	−	−	−	−	+	+	+	−	−	+	+	+	−	−	+⊥	−	−	−	−
	21	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+⊥	−	−	−	−
	28	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	++	−	−	−	−
	35	−	−	−	−	−	−	+⊥	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Pigeons	7	−	−	−	−	−	−	++⊥	+	+	−	−						−	+⊥	⊥	⊥	⊥
	14	−	−	−	−	−	−	++	+	+	−	−	++	+	+	−	−	+	+	⊥	⊥	−
	21	−	−	−	−	−	−	++	+	+	−	−	+⊥	−	−	−	−	+⊥	+	⊥	⊥	⊥
	28	−	−	−	−	−	−	++⊥	+	+	−	−	++	+	+	−	−	+	+⊥	⊥	⊥	⊥

* Animal died spontaneously.
** Culture from liver: + = positive; − = negative.
G Extent of granuloma.
A Number of organisms in tissue.
S Small forms of *Histoplasma* measuring less than 5 μ in greatest diameter.
M Middle-sized organism measuring from 5–10 μ in greatest diameter.
L Large forms of *Histoplasma* measuring over 10 μ in greatest diameter.

− Negative or absent.
+ Minimal or rare.
++ Slight or few.
+++ Moderate.
++++ Marked or numerous.
⊥ Intermediate sign.

pension, except rabbits and pigeons, which were injected intravenously with 0.1 ml of the suspension.

One guinea pig died spontaneously 3 days after the inoculation and 2 hamsters died 21 days and 95 days after the inoculation, respectively. Animals were killed and autopsies performed as listed in TABLE I. Fragments of tissue from the liver were cultured on Sabouraud's dextrose agar at room temperature and were observed for 6 weeks.

Organs were fixed in 10% formalin solution. Paraffin sections were stained with hematoxylin and eosin, and by the Gridley and Grocott procedures.

Additional tissue sections from 10 hamsters and 10 mice which had been inoculated intraperitoneally with 0.5 ml of saline suspension (1.8×10^6 cells of yeast phase per ml) of *H. duboisii* strain 44 were used for comparison. The animals had been killed on the 4th, 7th, 14th, 21st, 28th, 41st, 49th, 56th, and 63rd day after inoculation.

Tissue response and morphology of the organisms in omentum, liver, spleen, and lung were compared. The outline of the organisms was drawn with Abbe's camera lucida.

RESULTS

Guinea Pigs.—Three days after inoculation, only in the omentum was a minimal histiocytic reaction noted. Organisms were present in histiocytes. They varied in size (2–8 μ), stained faintly with hematoxylin, and were surrounded by a clear "halo" which measured about 1.5 μ in width. With the Gridley stain, cell walls of the organisms stained reddish-purple and had a distinct single contour. The cytoplasm of the organisms stained in a paler reddish-purple hue than the cell wall and was generally homogeneous. Other cells had a round "internal cytoplasmic structure," consisting of a loose reticular network of reddish-purple granules, with an indistinct outline.

Seven days after inoculation, moderate histiocytic reaction accompanied by a slight lymphocytic and polymorphonuclear leucocytic infiltration was observed in the omentum. The size of the organisms varied more than in the earlier stage, and forms measuring over 10 μ were observed. Most of the organisms were oval in shape, some were spherical or guitar-shaped and budding was observed occasionally (FIG. 1). A clear halo surrounded the organisms in hematoxylin eosin stains; most were weakly basophilic with distinct contour with a centrally located single basophilic granule. Others stained faintly basophilic or even acidophilic and had indistinct outlines. In the liver, a few minute granulomas of proliferated Kupffer cells were observed.

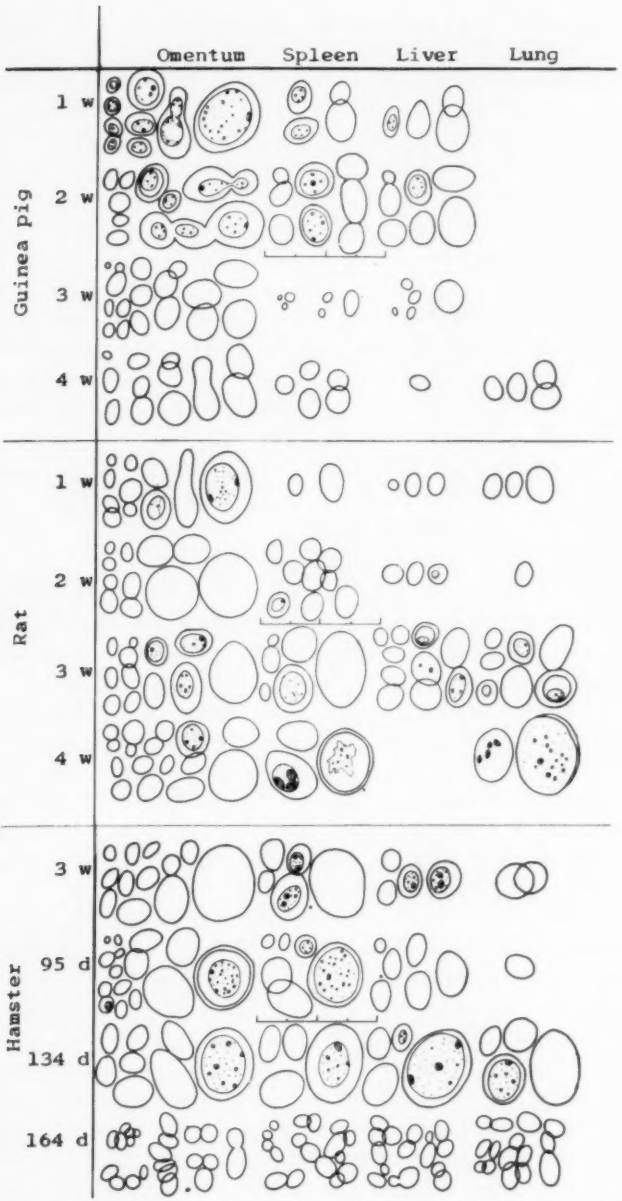

FIG. 1. *Histoplasma duboisii* in tissue of experimentally infected guinea pigs, rats and hamsters.

Two weeks after inoculation, a few small histiocytic foci were found in some alveolar septa. No organisms could be found in these foci. Three weeks after inoculation, coccoid minute organisms measuring 0.5–1.5 μ were found in the Kupffer cells and in the reticulum cells of the spleen (FIG. 3). With Gridley's stain, they were reddish rather

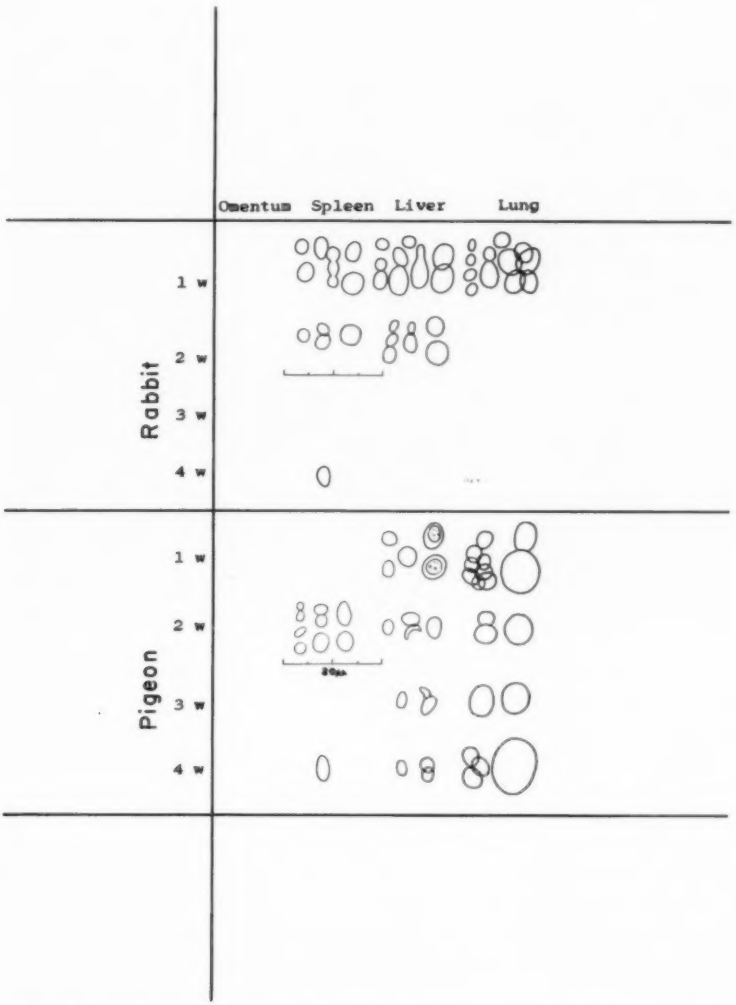

FIG. 2. *Histoplasma duboisii* in tissue of experimentally infected rabbits and pigeons.

than purple and were not digested by saliva; all were located within phagocytes. Four weeks after inoculation, large forms of the organisms were found only in the omental granulomas.

The tissue reaction of this group was essentially histiocytic and giant cell formation was not observed. The findings in different organs are shown in TABLE I and FIGURE 1. Great variations in the size of the organisms were observed except in the lung, where only small organisms were found.

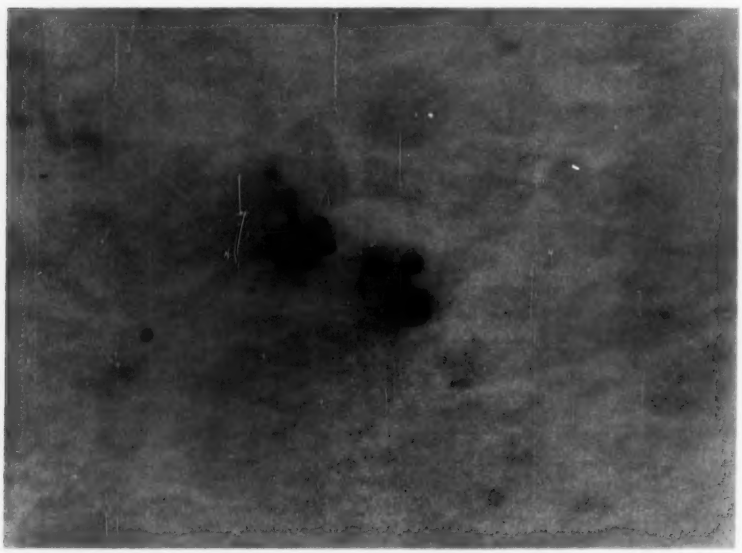

FIG. 3. *Histoplasma duboisii.* Coccoid minute organisms measuring 0.5 to 1.5 μ in greatest diameter in Kupffer cell. Guinea pig, 21 days after inoculation, liver. Grocott stain, $\times 2100$.

Rats.—In the early stages of the infection, histiocytic granulomas accompanied by lymphocytes, eosinophils and polymorphonuclear leucocytes were observed in omentum, spleen, liver and lung. Two to three weeks after the inoculation the tissue reaction was essentially an epithelioid cell granuloma. Four to five weeks after the inoculation, some of the organisms showed a double-contoured, somewhat refractile cell wall (TABLE I and FIG. 1). Occasional giant cells were present in the omental granuloma of a single rat after 74 days.

Hamsters.—The tissue reaction was essentially histiocytic in nature with a remarkable tendency to form giant cells and epithelioid cell

granulomas. The early reaction has been described previously (18), therefore only the late reaction will be mentioned here.

Three hamsters (95, 134, and 164 days after inoculation) showed almost identical findings with histiocytic and giant cell granulomas in all organs (FIG. 4). The vast majority of organisms was surrounded by a clear halo often within huge giant cells and histiocytes. Some of the

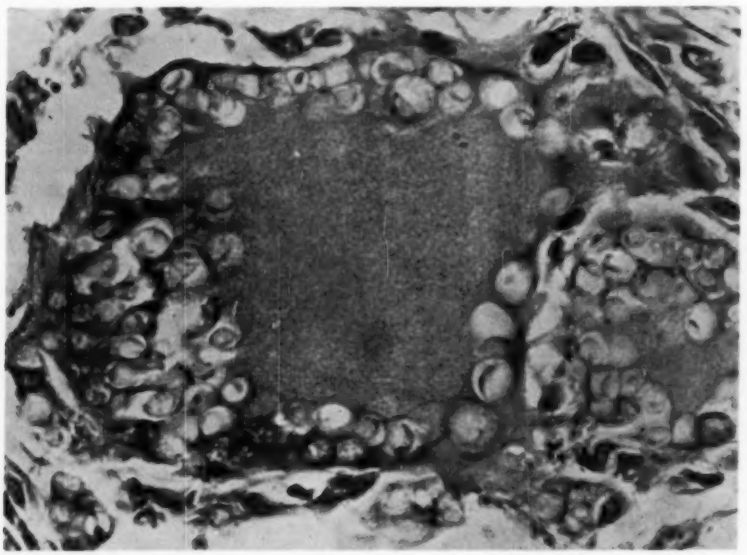

FIG. 4. *Histoplasma duboisii.* Enormous giant cells (180 μ in greatest diameter) containing large round to ovoid yeast cells. The yeast cells were surrounded by a clear halo and some show double-contoured wall. Hamster 134 days after inoculation, omental granuloma. Hematoxylin and eosin stain, × 600.

organisms had a refractile double-contoured cell wall and exhibited an internal (FIG. 5), ovoid structure with a reticular network on Gridley's stain.

One hundred and sixty-seven days after the inoculation, the giant cell reaction was not remarkable but histiocytic and epithelioid cell granulomas were observed. The organisms were small (up to 5 μ in greatest diameter). No large forms of the organisms were found. In this instance the organisms were indistinguishable from *H. capsulatum* (TABLE I and FIG. 1).

Rabbits.—Tissue reaction in rabbits was characterized by small and scarce histiocytic and epithelioid cell granulomas. Minimal periportal

histiocytic infiltration and small numbers of minute granulomas were found in the liver. In the splenic follicles and pulmonary alveolar septa, several small epithelioid cell granulomas were observed. No omental lesions were seen.

Only a few small and middle-sized organisms under 10 μ were found (TABLE I and FIG. 2).

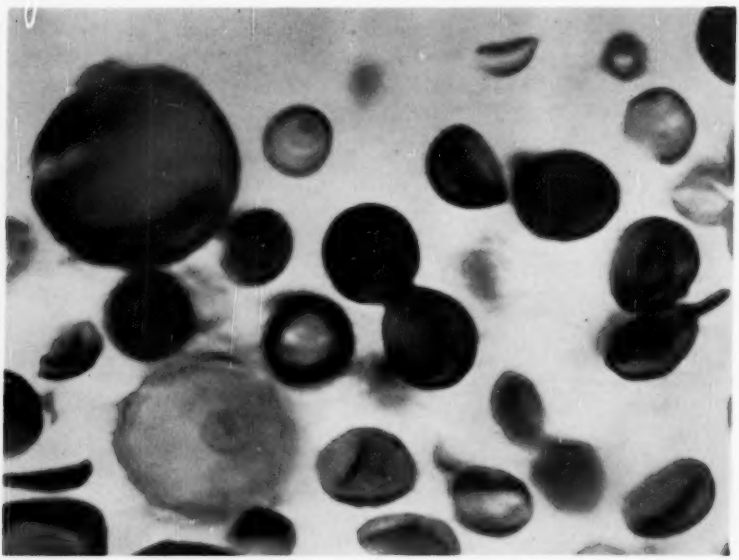

FIG. 5. *Histoplasma duboisii.* Conspicuous variation in size of the organisms. Large organisms with double-contoured wall and cytoplasmic reticular structure. Crescent forms of the organisms are also recognizable. The crescent-shaped organisms represent shells of collapsed yeast cells and can be most helpful in differentiating isolated yeast cells from artifacts. Hamster, 134 days after inoculation, omental granuloma. Grocott stain, ×2100.

Pigeons.—Tissue reaction in pigeons was essentially similar to those of rabbits. The size of the organisms varied as indicated in TABLE I and FIG. 2.

Cultural Results of the Livers. As indicated in TABLE I, the number of organisms in the liver was relatively small, and positive cultures were only obtained from livers with a relatively large number of organisms.

A previous cultural study of *H. duboisii* infection in hamsters and in mice, 66 days after the inoculation gave uniformly positive results (**16**); no attempts of culture were made in hamsters in the present series.

H. duboisii only exceptionally produced fatal infection in hamsters, but was not lethal for guinea pigs,[3] rabbits, rats or pigeons. The tissue reaction was principally histiocytic and giant cell reaction predominated in hamsters and mice. Epithelioid cell reaction was remarkable in rats, rabbits, and pigeons. The organisms varied conspicuously in size and small (under 5 μ), middle-sized (5–10 μ) and large (over 10 μ) forms were observed simultaneously in all animal species regardless of the stage of the infection, except in rabbits, in which no large organisms were found. The yeast cells were mostly oval in shape and no hyphal elements were noticed.

DISCUSSION

Brandt (**2**) reported that a South African strain of *H. capsulatum* (supposed to be *H. duboisii*) failed to produce systemic infection in guinea pigs, gerbils, mice or cats. Vanbreuseghem's experiments (**21**) suggested a low virulence of *H. duboisii* for mice, guinea pigs and rabbits. In our previous experiments, *H. duboisii* did not produce fatal infection in hamsters or mice (**18**). The present study also indicates that *H. duboisii* is an organism of low virulence to animals and scarcely produces fatal infection. The classical *H. capsulatum* is virulent for hamsters and uniformly produces fatal infection (**19**). Therefore the low mortality of *H. duboisii* in animals is quite remarkable in contradistinction to *H. capsulatum*.

Coccoid small particles, 1 to 2 μ in size, were also noticed in the cytoplasm of Kupffer cells and reticulum cells of the spleen. They were round to oval in shape and were not digested by saliva. They may represent organisms undergoing degeneration and disintegration, since transitional forms from small organisms to these small particles could be found.

With rare exceptions, in experimental infection and in spontaneous human disease caused by *H. capsulatum* the size of the organisms was 2–5 μ in diameter (**1, 3, 5, 11, 12, 17, 25**). Therefore, the constant and simultaneous occurrence of small, middle-sized and large forms of the organisms in *H. duboisii* infection permits differentiation of *H. duboisii* from *H. capsulatum*.

Both *H. capsulatum* and *H. duboisii* are uninuclear (**10**). However, the peculiar cytoplasmic structure, composed of a loose reticular network of reddish-purple granules in the Gridley stain, and a distinct, refractile cell wall were often found in yeast cells of *H. duboisii* but never in *H. capsulatum*.

[3] The guinea pig, dead 3 days after inoculation, expired from intercurrent disease.

In experimental infection with *H. duboisii*, especially in hamsters and in mice, some of the giant cells were as large as 220 $\mu \approx$ 500 μ, and contained a huge number of organisms (**18**).

In some of the experiments with *H. capsulatum* in hamsters, giant cell reaction was also observed, but the giant cells were generally scarce and less than 60 μ in diameter. Only small forms of the organisms or laminated basophilic bodies (Schaumann bodies) could be found in their cytoplasm. Schaumann body formation in the hamster is a nonspecific reaction elicited by heterogeneous organisms. Experimental infection in mice with *H. duboisii* resulted exceptionally in the formation of Schaumann bodies (**14**), which was never seen in mice infected with *H. capsulatum*.

H. duboisii possesses the following biologic properties: 1) low virulence for animals, 2) simultaneous occurrence of small, middle-sized, and large forms of yeast cells in tissue, 3) a double-contoured cell wall and "internal structure," 4) marked tendency to form huge giant cells, especially in hamsters, and 5) Schaumann bodies in mice. Differences in amino-acid requirements and collodion particle agglutination tests between *H. duboisii* and *H. capsulatum* have been also reported (**4, 13**).

SUMMARY

Experimental infection with *H. duboisii* was studied periodically in guinea pigs, rats, hamsters, rabbits, and pigeons.

From the results obtained, *H. duboisii* is believed to possess biologic and morphologic properties different from *H. capsulatum*. Therefore, we believe that *H. duboisii* can be separated from *H. capsulatum* and is not a simple variant of *H. capsulatum*.

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A NEW EMERICELLOPSIS FROM WISCONSIN, WITH NOTES ON OTHER SPECIES [1]

M. P. Backus and P. A. Orpurt[2]

(with 15 figures)

In the course of ecological studies on the soil fungi of Wisconsin prairies, conducted between 1952 and 1954 (**16**), a distinctive organism belonging to the Eurotiaceae was repeatedly isolated from a wet prairie in the University of Wisconsin Arboretum at Madison and was again encountered several times in soil samples taken from wet prairies in Iowa County, Walworth County, and Marquette County. In all, one hundred nine isolates were obtained. This organism, initially catalogued as "WSF 47," was eventually determined to be an undescribed fungus belonging to the genus *Emericellopsis* van Beyma.

Although *Emericellopsis* was poorly known in mycological circles at the time that the above-mentioned isolations were made, it has since attained considerable prominence. The genus was erected by van Beyma (**20**) in 1940 for certain fungi that had been isolated from soil in Holland. In van Beyma's article the type species, *E. terricola*, was fully described; in addition, a variety *"glabra"* was very briefly characterized. Apparently the genus was not reencountered in nature until about a decade later, when Malan (**13**) obtained an isolate from vineyard soil in Italy. This latter investigator, presumably unaware of van Beyma's publication, erected the new genus *Peyronellula* for his fungus and named the species *P. mirabilis*. Stolk (**19**) in 1955 transferred Malan's organism to *Emericellopsis* and added a third species, *E. minima*, isolated from African soil. The article by Stolk helped greatly to acquaint mycologists with this interesting but hitherto obscure genus. A fourth species, found in Canada, was described by Cain (**3**) in 1956 as *Saturnomyces humicola*, the type of still another new genus in the Eurotiaceae. The transfer to *Emericellopsis* was in this case accomplished by Gilman (**9**). In 1957 a fifth species, *E. salmosynnemata*, was described by Grosklags and Swift (**11**), who had encountered it as the perfect stage of the well-known anti-

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biotic-producing organism *Cephalosporium salmosynnematum* Roberts. Grosklags and Swift tested the other known species and varieties of *Emericellopsis* for antibiotic production and reported that *E. terricola* var. *glabra* and "WSF 47", like *E. salmosynnemata*, are strong and consistent producers of an antibiotic or antibiotics inhibitory to *Staphylococcus aureus* (avian strain), *Salmonella typhimurium*, and *Salmonella gallinarum*. In contrast, they found *E. minima*, *E. mirabilis*, *E. humicola*, and *E. terricola* (var. *terricola*) to be weak and sporadic producers.

After the discovery of the perfect stage of *Cephalosporium salmosynnematum*, all available isolates of *Emericellopsis* were subjected to further study in the antibiotics laboratory of Professor J. F. Stauffer and the senior author at the University of Wisconsin. From this investigation, which included extensive submerged fermentation tests, it became clear that the type culture of *E. terricola* var. *glabra* was a superior producer of cephalosporin (synnematin)—the yields obtained from it being even substantially better than those secured from two improved mutant strains of *E. salmosynnemata* which had been developed at the Michigan Department of Health, in Lansing, Michigan. Kavanagh et al. (**12**) also tested the various available *Emericellopsis* isolates; and their results in general confirmed the report of Grosklags and Swift regarding antibiotic production by the different species and varieties, but the substantial superiority of *E. terricola* var. *glabra* as a source of cephalosporin N (= synnematin B) was in evidence in the fermentations which they conducted. More recently Elander (**8**) has shown that the type culture of *E. terricola* var. *glabra* and "WSF 47" produce, in addition, an antibiotic tentatively identified as cephalosporin C. Cephalosporin C was originally described in England (**15**) from culture filtrates of "*Cephalosporium acremonium*" (Brotzu strain) and is potentially a very valuable drug, since it is active against penicillin-resistant strains of *Staphylococcus aureus*.

Routien (**18**) in 1957 recorded the isolation of "*E. mirabilis*" from Indiana soil, but this appears to be an erroneous report. Grosklags (**10**) and the writers have examined the Routien isolate and agree that it is best assigned to *E. terricola* var. *terricola*. Durrell (**7**) also identifies it as *E. terricola*. However, Grosklags (**10**) has shown that the Routien isolate is a relatively good producer of synnematin, whereas the type culture of *E. terricola* var. *terricola* definitely is not. Two other records of recent isolations of *Emericellopsis* have appeared in the literature. From soil in a Rocky Mountain area in western United States Durrell (**7**) repeatedly obtained an *Emericellopsis* which he determined as *E. terricola*. This fungus has not been examined by the writers. Finally, Warcup (**21**) found an *Emericellopsis* in soil of a wheat field at the Waite

Agricultural Institute in South Australia. Warcup, like Routien, identified his organism as *E. mirabilis;* but again this seems to be an erroneous determination as will be explained below. Numerous isolates of *Emericellopsis* have also recently been obtained in Holland by workers at the Centraalbureau voor Schimmelcultures at Baarn (personal communication from Dr. A. Stolk of that institution).

From the account given, it must be apparent that in the short span of time since 1955 *Emericellopsis* has become a well-known taxon. This stems in part, as has been pointed out, from Stolk's 1955 publication, which appeared in a widely distributed mycological journal; in part from the greatly increased activity in the field of soil mycology during the past decade; and especially from the work of Grosklags and Swift, which first made clear that *Emericellopsis* is a genus of antibiotic producers. At the University of Wisconsin various members of the genus have figured in physiological and biochemical studies (**8, 14**), and *E. terricola* var. *glabra* is currently being used in a strain development program. The latter program, which thus far has closely paralleled the one earlier carried out with great success on *Penicillium chrysogenum* (**2**) in the same laboratory, has already yielded UV-induced mutants of considerably enhanced capacity to synthesize the antibiotic (**8**). Elsewhere cytological and other studies on *Emericellopsis* are in progress. The genus has also become well-known in certain industrial laboratories.

Emericellopsis is a well-defined taxon in the Eurotiaceae. The cleistothecia have a transparent peridium through which the scattered subglobose asci may be seen within the unopened fructification. The ascospores are one-celled, typically ellipsoid, dark-walled, and provided with three to six longitudinal wings. The smallest ascocarps contain only one or two asci, but the largest have a hundred or more. When viewed with the naked eye or under low magnification, the fructifications have a black or dark brown color; but this color is conferred almost entirely by the spores, as can readily be seen at higher magnifications or when a fruiting body is broken open. All species have a *Cephalosporium* imperfect stage.

On the basis of ascospore size, the various described species can conveniently be divided into three categories. *E. minima* and *E. humicola* have small ascospores, mostly 5.0–6.0 μ in length; a second group, to which *E. terricola* var. *terricola, E. terricola* var. *glabra,* and *E. salmosynnemata* belong, has spores of intermediate length, mostly 7.5–10.0 μ; finally there is the large-spored form, *E. mirabilis,* where, according to our measurements, ascospore length averages about 12.5 μ and occasionally exceeds 15 μ.

The Wisconsin wet prairie entity belongs to the small-spored section

of the genus, with ascospores in the general size range of those of *E. humicola* and *E. minima*. Although the ascocarps of the Wisconsin organism closely resemble the fructifications found in *E. humicola*, the ascospores differ appreciably in both shape and wing characters. There is a closer resemblance to *E. minima* in ascospore morphology; however, as is explained below, some important considerations make it impossible to assign the Wisconsin fungus to that taxon. The *Emericellopsis* from Wisconsin wet prairies is therefore described as a new species.

Emericellopsis microspora Backus & Orpurt, sp. nov.

Cleistothecia globosa, glabra, brunneonigra, 18–120 μ (plerumque 30–60 μ) in diametro; peridium cleistothecii translucidum. Asci globosi vel subglobosi, incomposite dispositi, octospori, 9–12 μ, evanescentes. Ascosporae continuae, ellipsoideae vel ovoideae, olivaceae, plerumque 5.0–6.2 × 3.2–3.8 μ, frequenter cum gutta oleagina et cum 3–4 alis hyalinis saepe triangularibus 1.5–4.0 μ altitudine per longitudinem extantibus.

Status conidicus: *Cephalosporium*.

Conidiophorae hyalinae, plerumque non ramosae, plerumque 25–60 × 2–3 μ; conidia hyalina, ellipsoidea vel ovoidea, continua, 3.0–10.0 × 1.5–4.0 μ.

E solo palustri, Wisconsin, U. S. A.

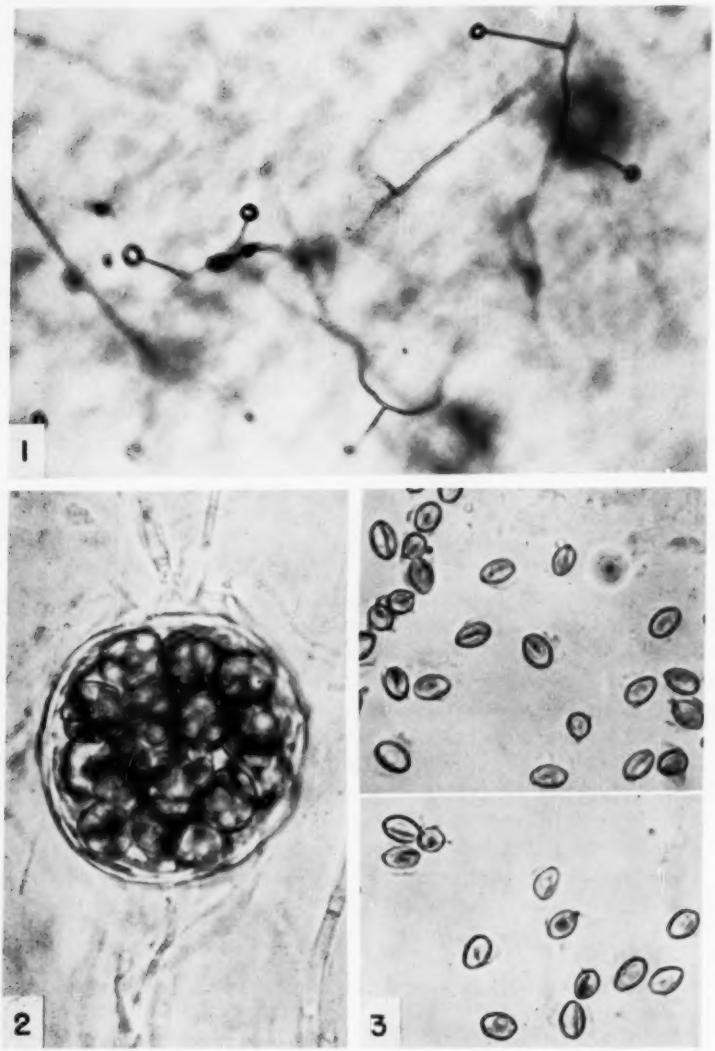
Ascocarps dark, astomatous, globose, glabrous, 18–120 μ (mostly 30–60 μ) in diameter; wall of the cleistothecium subhyaline, transparent; asci 8-spored, 1–many per fructification, scattered, subglobose to globose, 9–12 μ in diameter, with a thin, evanescent wall. Ascospores broadly ellipsoid to ovate, one-celled, pale greenish brown in color, most 5.0–6.2 × 3.2–3.8 μ (average about 5.6 × 3.5 μ), commonly provided with an oil drop and bearing 3 (occasionally 4) delicate hyaline longitudinal wings that are variable in shape but often triangular, projecting out 1.5–4.0 μ from the body of the spore.

Conidial stage: *Cephalosporium*.

Conidiophores hyaline, arising from the substrate or occasionally from arching aerial hyphae, mostly unbranched, usually septate near the base, mostly 25–60 × 2–3 μ. Conidia 1-celled, hyaline, 3.0–10.0 × 1.5–4.0 μ, ovoid to ellipsoid.

Homothallic; ascigerous stage obtained from single ascospores and from single conidia.

TYPE material has been deposited in the Herbarium of the University of Wisconsin, the Farlow Herbarium at Harvard University, the collection at the Commonwealth Mycological Institute, Kew, Surrey, England, and in the cryptogamic herbarium of the Museum National d'Histoire Naturelle, Paris. Culture "WSF 47" has been deposited at the Ameri-



FIGS. 1–3. *Emericellopsis microspora*. 1. *Cephalosporium* stage. Surface view of a cornmeal agar plate culture, showing conidiophores and conidial heads, × 155. 2. Whole ascocarp, × 850. Asci are visible through the transparent peridium. 3. Ascospores, × 850.

can Type Culture Collection, Washington, D. C., and at the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

Colonies grow well on Czapek-Dox, potato-dextrose, honey-peptone, malt extract, oatmeal, and cornmeal agars. On all of these media except cornmeal agar the mycelial mat is pale to bright salmon pink; on all except oatmeal and cornmeal agar it is radially folded. Colonies on Czapek-Dox agar in Petri dishes reach a diameter of approximately 40 mm after incubation in the light for two weeks at a temperature of 26°–28° C. On cornmeal agar the mycelium is mostly submerged and essentially colorless; but cultures on this substrate eventually darken as a result of the production of vast numbers of tiny ascocarps throughout the medium. Colonies on oatmeal agar and potato-dextrose agar blacken in the center as a result of even more profuse development of ascocarps. On all media used in these studies the growth rate of *E. microspora* was found to be intermediate between that of the other two small-spored species of *Emericellopsis*, and this is in agreement with the report by Grosklags and Swift (11). Colonies of *E. humicola* are substantially smaller and those of *E. minima* much larger than those of *E. microspora* after an incubation period of one or two weeks. Color differences in the mycelial mat also serve to differentiate the three entities when cultures on such media as Czapek-Dox, honey-peptone, or potato-dextrose agar are incubated at ordinary room temperatures; for, whereas the colonies of *E. microspora* are salmon pink, those of *E. humicola* are grey and the ones of *E. minima* are bright golden yellow.

Production of conidia is relatively sparse in *E. microspora* and the conidial stage is not very distinctive. The conidiophores, which arise from the substrate mycelium or from arching aerial hyphae (FIG. 1), are somewhat variable in height, up to 95 μ but mostly 25–60 μ; and, as in many other Cephalosporia, the spores are highly variable in size and shape. In both of the other species of *Emericellopsis* with small ascospores, the conidiophores are somewhat shorter, although, according to our observations, some exceeding the published dimensions can readily be found in cultures of these entities. As Grosklags and Swift (11) have already pointed out, production of conidia in the type culture of *E. minima* is much more profuse than it is in either *E. microspora* (=WSF 47) or *E. humicola*.

As well as can be judged by the behavior of available isolates, the various *Emericellopsis* entities differ greatly in their retention of ability to produce the perfect stage after cultivation in the laboratory for various periods of time. Thus, the transfers from the type cultures of *E. terricola* var. *terricola* and *E. minima* which the writers obtained through the

courtesy of Dr. C. R. Benjamin, then of the Northern Regional Research and Development Division, U. S. Department of Agriculture Research Service, at Peoria, Illinois, had almost completely lost their ability to fruit when received in 1956. A culture of *E. humicola* which was secured from Dr. R. F. Cain later the same year and which fruited profusely for about eighteen months has now become completely sterile. *E. salmosynnemata* after being maintained for six years on agar in our laboratories fruits less readily than it did at first. In contrast, *E. microspora* has retained its fertility tenaciously; so have the types of *E. mirabilis* and *E. terricola* var. *glabra*—the latter after more than twenty years of repeated transfers.

Dodge's cornmeal agar, prepared as described by Grosklags and Swift (**11**), appears to be an ideal medium for production of the perfect stage of all known species of *Emericellopsis*. Oatmeal agar (**10**) is also a very favorable substrate for obtaining ascocarps of several species. On media such as Czapek-Dox agar, potato-dextrose agar, and honey-peptone agar (**11**) most species of this genus make a fine vegetative growth but form few if any cleistothecia. Among all the isolates of *Emericellopsis* available to the writers, those of *E. microspora* have been unique in that they usually fruit profusely on potato-dextrose agar and sometimes moderately well on honey-peptone agar. Stolk (**19**) indicated that *E. minima* "readily produces perithecia on most media"; this, however, could not be verified since, as was pointed out above, the only available culture of this species had very nearly completely lost its ability to fruit on any medium. In all species of *Emericellopsis*, ascocarps develop down in the agar as well as the surface. In the case of *E. microspora* growing on cornmeal agar, the tendency for submerged fruiting is especially strong. All species of *Emericellopsis* have also been shown to be homothallic (**7, 10**). The first report of homothallism in any species of the genus was that by Grosklags and Swift (**11**) in the case of *E. salmosynnemata*. Grosklags (**10**) reported that *E. microspora* (=WSF 47) is homothallic, and this has been verified by the writers.

Ascocarps of *E. microspora* (FIG. 2) have been observed to vary from 18 μ to about 120 μ in diameter. Most, however, fall within the range of 30–60 μ. Fructifications as large as 90–120 μ in diameter have been seen only a few times. Very minute ascocarps, containing from one single ascus to perhaps a half dozen asci, are not at all uncommon in this species. On the whole, ascocarp size seems to be a feature of limited value for speciation in the genus under consideration. The fructifications of *E. humicola*, *E. salmosynnemata*, *E. terricola* var. *terricola*, and *E. terricola* var. *glabra* are of approximately the same size and superficially of the same

appearance as those of *E. microspora*. In agar cultures they appear as tiny black dots just visible to the naked eye. There is abundant evidence, however, that in *E. mirabilis* and *E. minima* cleistothecia grow to a larger size than in other members of the genus. On the same cornmeal agar substrate on which *E. microspora* produces ascocarps seldom larger than 70 μ in diameter, many up to 130 μ in diameter and a few up to 185 μ in diameter were found in cultures of *E. mirabilis* by Grosklags and Swift (**11**); and similar observations have been made repeatedly in the present study. Indeed, the ascocarps of this organism are consistently so much larger than those of any other species which we have seen in good fruiting

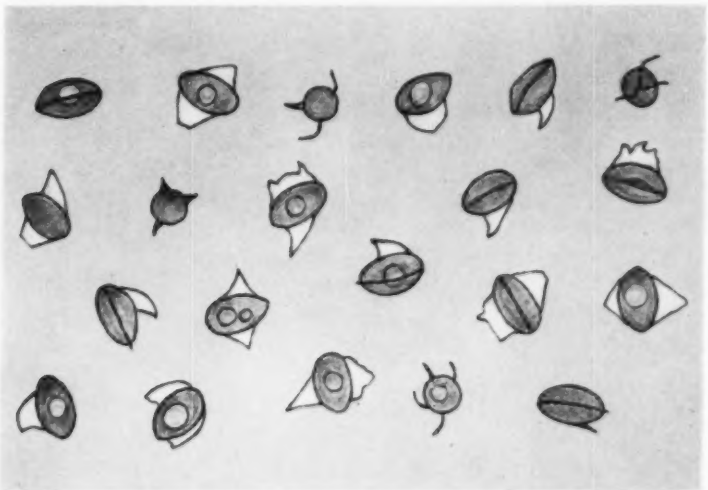
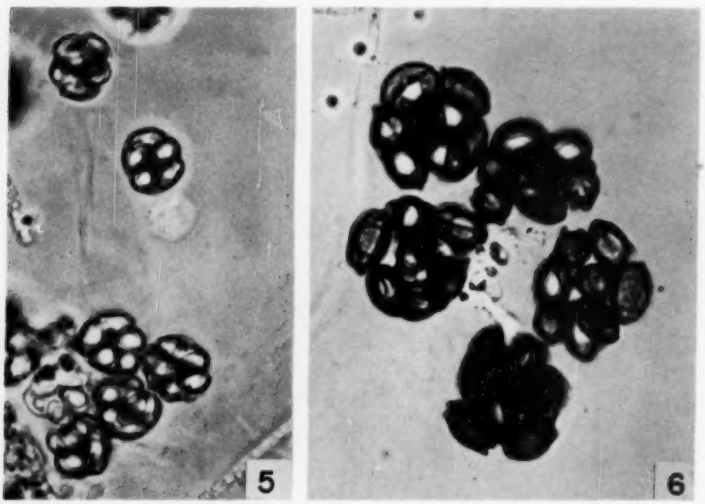


FIG. 4. *Emericellopsis microspora*. Ascospores, showing detail of wings. ×1600.

condition that both plate and tube cultures of it on cornmeal agar are immediately distinguishable upon the most casual observation. In describing *E. minima*, Stolk (**19**) gave the ascocarp size as 90–300 μ, and Grosklags and Swift (**11**) observed fructifications up to 200 μ in diameter in this species. The large size attained by ascocarps in this taxon is of special interest here, since, on the basis of microscopic characteristics, *E. minima* is the only species with which *E. microspora* could possibly be confused.

Ascus walls in *E. microspora* are fragile and frequently break down as the ascocarps age. Asci (FIGS. 2, 5) are only 9–12 μ in diameter—scarcely as large as one single ascospore in *E. mirabilis* (FIG. 10). As has already been indicated, the ascospores (FIGS. 3, 4, 7) resemble those

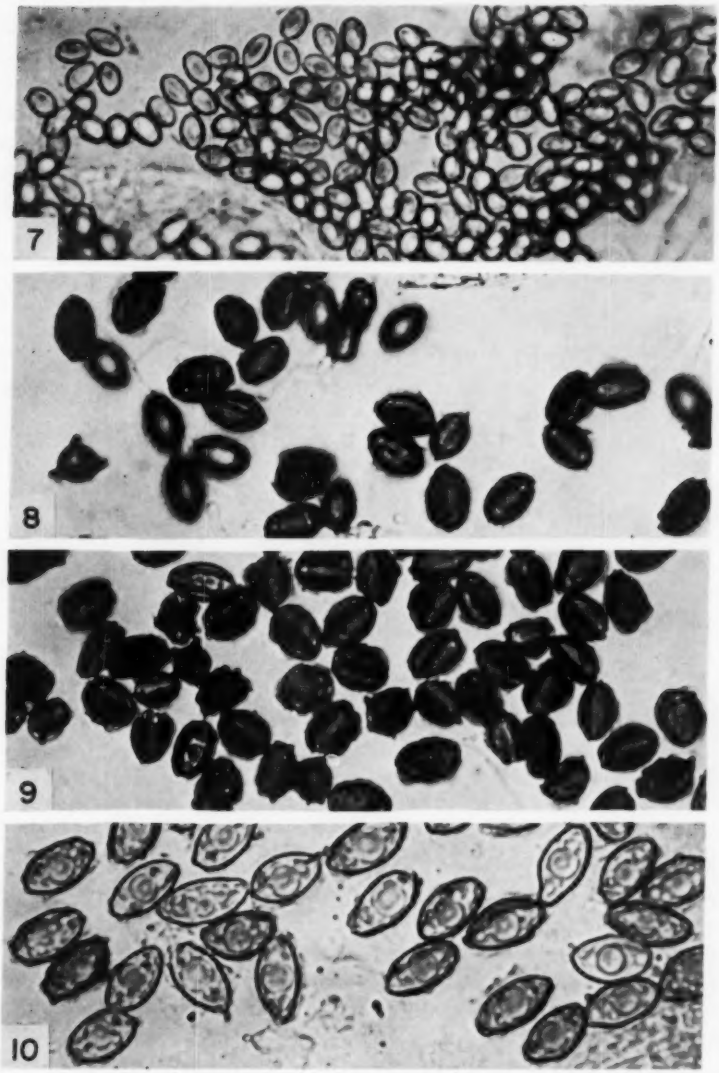
of *E. minima* to a considerable extent. They are of approximately the same size and shape, and the form of their wings is most like that encountered in the latter species. In *E. microspora* these appendages are very delicate and transparent; they are variable in form but often are more or less triangular, with either a sharp or truncate tip. When the spore is seen in end view, the wings frequently look like delicate whip-like outgrowths. However, in no case have they been observed to extend out more than 4 μ from the body of the spore. They are definitely less conspicuous structures than the wings of *E. minima*, which Stolk de-



FIGS. 5 AND 6. 5. Asci of *Emericellopsis microspora*, ×850.
6. Asci of *E. glabra*, ×850.

scribed as being 3.5–6.0 μ long. *E. humicola* is readily distinguished from both of the other small-spored species by the more slender form of its ascospores and by its shorter wings which also are rarely triangular. The ascospores of *E. humicola* (average $5.4 \times 2.6\ \mu$, according to our measurements) have a smaller volume than do ascospores of any other described species of *Emericellopsis*.

On a few occasions, a small number of ascospores substantially larger than normal (e.g., $7.5 \times 4.0\ \mu$; $8.0 \times 5.0\ \mu$, etc.) have been observed in *E. microspora*. These "giants" appear to be abnormalities, and twice they were found in asci containing only three and four spores. Such giant spores have been reported in other Ascomycetes (**1, 4, 5, 6**). A very



FIGS. 7–10. Ascospores of three species of *Emericellopsis* photographed at the same magnification. All × 850. 7. *E. microspora.* 8. *E. glabra* (type). 9. *E. glabra* (Australian isolate, "T 15"). 10. *E. mirabilis.*

few spores of unusually small dimensions (ca. $3.5 \times 2.5\ \mu$), but apparently fully mature, have likewise been encountered in *E. microspora*. The rarity of these "dwarfs" suggests that they too should be regarded as abnormalities. Whether such minute spores will germinate has not been determined. After prolonged study of the spore situation in *Emericellopsis*, the writers have come to the conclusion that the usual or average size of ascospores is of great taxonomic significance but that to emphasize the absolute range, which would take in these abnormalities, only confuses the issue of speciation.

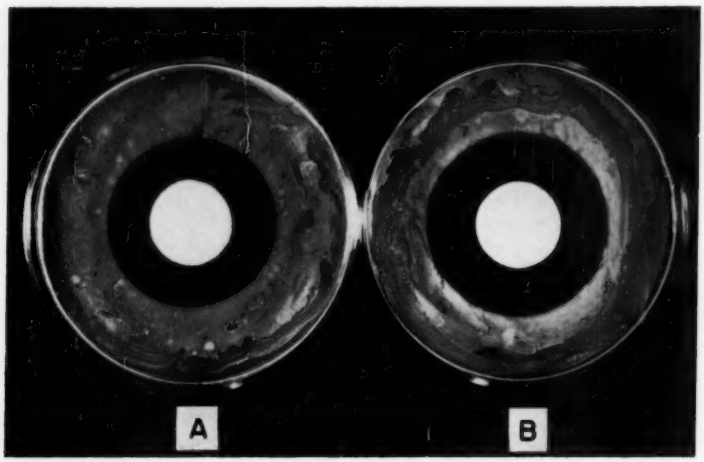


FIG. 11. Inhibition of *Staphylococcus aureus* by colonies of *Emericellopsis salmosynnemata* (A) and *E. microspora* (B). The colonies were started from hyphal tips and grown on a nutrient agar of neutral pH. After about a week, a suspension of *Staph. aureus* was painted over the entire exposed agar surface of each culture and the plates were then incubated at 37° C for 15 hours in an inverted position. The clear inhibition zones around the colonies were caused by the antibiotic which had diffused out into the agar from the mycelium.

Antibiotic production by *E. microspora* (under the designation "WSF 47") has been investigated by Grosklags and Swift (**11**), Kavanagh et al. (**12**), Elander (**8**), and Grosklags (**10**). FIGURE 11, B, shows the inhibition of *Staphylococcus aureus* (avian strain) by a colony of *E. microspora* which has been grown on a medium containing 15.0 g agar, 5.0 g Difco bacto-peptone, 3.0 g Difco yeast extract, and 1.0 g cerelose per liter. In contrast with the very strong antibiotic production exhibited by this species, only very weak production has ever been reported for either of the other two small-spored members of the genus, *E. humicola* and *E.*

minima. Results of the writers' tests on the latter two entities are in complete agreement with what others have found.

It is a matter of considerable interest that *Emericellopsis microspora* was isolated from four different counties in Wisconsin but always from low prairies. In a ten-year study of the microfungal populations in undisturbed soils of the state, this organism has not been isolated from any other kind of habitat. It was not encountered in a survey of the southern hardwood forests of the state nor in an investigation of microfungi of the soils in the northern conifer-hardwood forests; neither was it found in mesic or dry prairies. Still further, and most surprising perhaps, it has not been found in any other lowland area thus far surveyed (southern floodplain forests, northern bog communities, northern and southern sedge meadows). *E. minima*, which seems to be the species most closely related to *E. microspora*, was isolated from mangrove swamp mud in East Africa. The other small-spored species of *Emericellopsis*, *E. humicola*, was encountered in isolations from peat soil in Canada.

As has already been indicated, studies of all available isolates of *Emericellopsis* and large numbers of spontaneous and induced variants were carried out over a period of years in the mycology and plant physiology laboratories at the University of Wisconsin. These studies have led to the conclusion that *E. terricola* var. *glabra* is both morphologically and physiologically substantially different from *E. terricola* var. *terricola*, and the former taxon is therefore here raised to the rank of a species.

Emericellopsis glabra (van Beyma) Backus & Orpurt, comb. nov.

Emericellopsis terricola var. *glabra* van Beyma. Antonie Van Leeuwenhoek ned Tijdschr. **6**: 266. 1940.

Van Beyma, in his very brief description of *E. terricola* var. *glabra*, pointed out the difference in wing characters between this organism and his "*E. terricola*" (*E. terricola* var. *terricola*), but he failed to state that the ascospores of *E. terricola* var. *glabra* are substantially larger. That was made clear by Stolk (**19**) and has been repeatedly verified by the writers and also by Grosklags (**10**). Grosklags, in a study of a large number of variants derived from the type culture, found that, however else they were modified, the variants did not lose their ability to form ascocarps on cornmeal agar and they were unchanged in ascospore morphology. The writers are in full accord with Grosklags (**10**), Grosklags and Swift (**11**), and Durrell (**7**) in their view that ascospore characters provide the best basis for speciation in this genus, and accordingly put considerable stress on the peculiarities of the ascospores in elevating van

Beyma's variety. The ascospores of *E. glabra* are both longer (average 9.7 μ) and wider (average 5.5 μ) than those of *E. salmosynnemata* and *E. terricola*, the other two species with spores of intermediate size; only *E. mirabilis* has spores that are bigger. The wings are distinct from those encountered on spores in other members of the middlesized spore group, being wider than those found in *E. salmosynnemata* and lacking the prominent dentate margin of the ones in *E. terricola*. The spore wall in *E. glabra* also shows a very finely punctate surface which is distinctive. This sculpturing is visible only under relatively high magnification. Spores of *E. salmosynnemata* commonly exhibit a similar but coarser sculpturing (**11**). In other species of *Emericellopsis* the wall appears to be nearly smooth. The type culture of *E. glabra* is remarkably stable. After more than twenty years of cultivation in the laboratory, it has lost little if any of its ability to produce ascocarps; populations grown from conidia are highly uniform; and the colonies show little tendency to sector (**10**). Studies have indicated that the type culture of *E. glabra* produces relatively large quantities of synnematin B (= cephalosporin N) and also an antibiotic active against penicillin-resistant *Staphylococcus aureus* (**8, 12**); in contrast, the type culture of *E. terricola* seems to have very little ability to elaborate any antibiotic material.

One of the very notable physiological traits of the type culture of *E. glabra* was pointed out by Grosklags (**10**), who found that this isolate makes essentially no growth on a modified Czapek-Dox agar in which dextrose is substituted for sucrose, whereas all other species of *Emericellopsis* grow on this medium and most of them grow very well. In recent experiments conducted by the senior author, *E. terricola*, *E. minima*, *E. humicola*, *E. salmosynnemata*, and *E. microspora* grew vigorously and formed a heavy mycelial mat both on Czapek-Dox agar and on modifications of this medium in which dextrose (reagent grade) or cerelose was substituted for sucrose (reagent grade). In contrast, *E. mirabilis* formed only a thin, although broadly-spreading colony on the Czapek-Dox substrate and grew more slowly on the modified substrate with dextrose or cerelose. Colonies of *E. glabra* on Czapek-Dox agar were likewise thin and spreading, while there was no measurable growth on the modified media after incubation for two weeks at 26°–29° C, either in the light or in darkness (FIG. 12). These results indicate a physiological kinship between *E. glabra* and *E. mirabilis* and they confirm Grosklags' report.

As pointed out above, Warcup in 1957 reported the isolation of a species of *Emericellopsis* from soil of a wheatfield at the Waite Agricultural Institute in Australia. Insofar as the writers have been able to

determine, this is the only report of *Emericellopsis* from that continent. Warcup (**21**) listed his organism as *E. mirabilis* and, in a private communication to one of us (Backus), indicated that the wheatfield from which the fungus had been obtained was periodically flooded.

In 1958 Dr. Warcup kindly supplied to the senior author a culture of his *Emericellopsis* bearing the number "T 15." This has been studied at the University of Wisconsin over a period of two years and it has

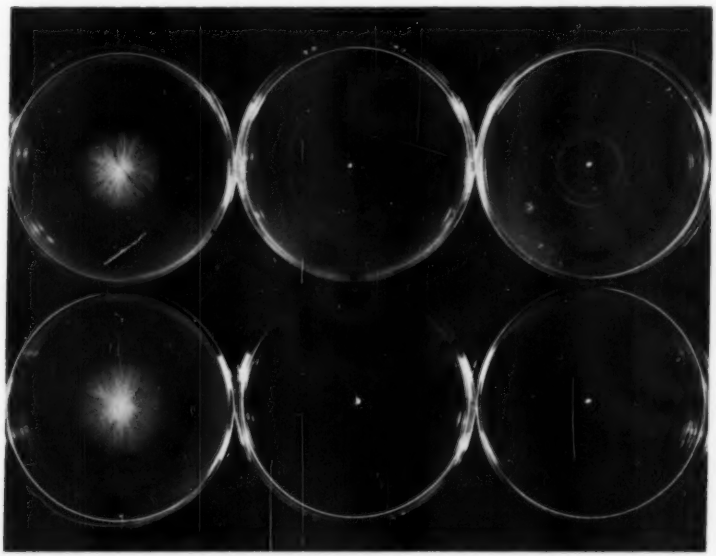


FIG. 12. Two isolates of *Emericellopsis glabra* on three different media. All cultures were incubated in the light for 2 weeks at 26°–29° C. Upper row: *E. glabra*, type. Lower row: *E. glabra*, Warcup strain T 15. Left: on Czapek-Dox agar. Middle: on modified Czapek-Dox agar (reagent grade dextrose substituted for reagent grade sucrose). Right: on modified Czapek-Dox agar (cerelose substituted for reagent grade sucrose).

been found to be a strong antibiotic producer (**8**). Morphological studies have led to the conclusion that this organism should be assigned to *Emericellopsis glabra*, principally because of the close similarity between its ascospores and those of the type of the latter species (FIGS. 8, 9). The similarity extends to size, shape, wing characteristics, and wall sculpturing. The ascospores of *E. mirabilis* (FIG. 10) are very substantially larger. After identification of the Australian fungus had been made on the basis indicated, additional confirmation was received when "T 15"

was grown on regular Czapek-Dox agar and on the modifications of it described above, for the colonies which developed on Czapek-Dox agar looked essentially identical with those of the type of *E. glabra* grown on the same medium under the same conditions, and neither organism showed any appreciable growth during two weeks of incubation on either of the modified Czapek's media (FIG. 12). In another experiment, a small drop of Pontecorvo's vitamin mixture (17) was added to plates

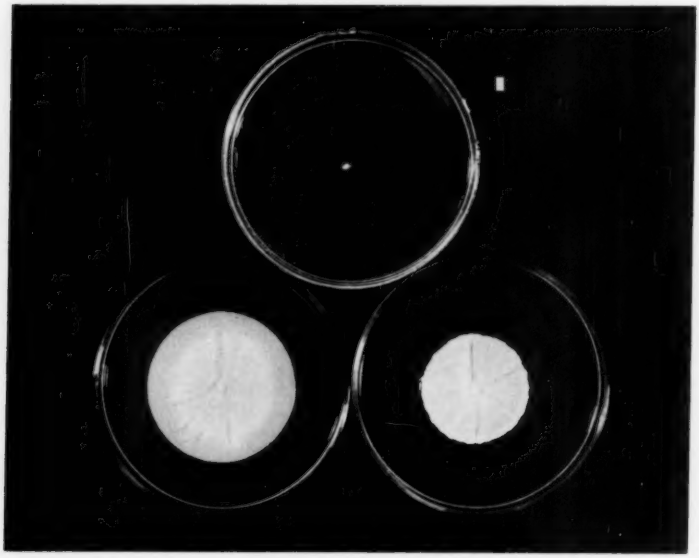


FIG. 13. *Emericellopsis glabra* (Warcup strain T 15) under various cultural conditions. All cultures were incubated for two weeks at room temperature, 26°–32° C. Upper culture: on modified Czapek-Dox agar (cerelose substituted for sucrose), incubated in the light. Lower left: on modified Czapek-Dox agar (cerelose substituted for sucrose) plus vitamins, incubated in the light. Lower right: on modified Czapek-Dox agar (cerelose substituted for sucrose) plus vitamins, incubated in the dark.

of modified Czapek's agar (reagent grade dextrose, or cerelose, substituted for sucrose) as they were poured; and on this substrate both the type of *E. glabra* and "T 15" grew readily, forming a heavy, pigmented mycelial mat (FIG. 13). Both organisms also responded in an identical manner to the addition of the Pontecorvo vitamin mixture to the regular sucrose-containing Czapek-Dox agar; each now formed a heavy, salmon-colored colony instead of the thin, colorless growth obtained on the un-

amended medium (FIG. 14, upper row of cultures). Still further support for the assignment of the Australian fungus to *E. glabra* is found in the fact that the antibiotic production pattern of "T 15" corresponds with that of the type of this species but not with that of *E. mirabilis* (**8**). The Australian culture produces conidia more abundantly than does the type of *E. glabra* and there are some cultural differences to be observed when

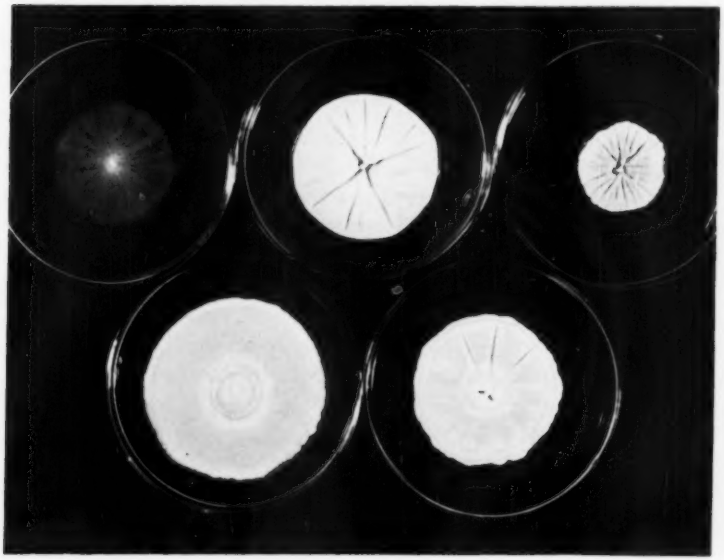


FIG. 14. Two species of *Emericellopsis* grown under various cultural conditions. Upper row: *E. glabra* (Warcup strain T 15): left, on Czapek-Dox agar, incubated in the light; middle, on Czapek-Dox agar plus vitamins, incubated in the light; right, on Czapek-Dox agar plus vitamins, incubated in the dark. Lower row. *E. salmosynnemata:* left, on Czapek-Dox agar, incubated in the light; right, on Czapek-Dox agar, incubated in the dark. All cultures were grown for 2 weeks at room temperature, 26°–32° C.

the two isolates are grown on media such as honey-peptone and potato-dextrose agars. But these differences are not regarded as critical. Ascocarp size is generally similar in "T 15" and the type of *E. glabra*, most fructifications in both cases falling in the range of 30 to 60 μ. However, a few cleistothecia measuring between 100 and 130 μ have been found in occasional cornmeal agar cultures of the Australian organism while none closely approaching this size have ever been encountered in this laboratory in the type culture of *E. glabra*. On the other hand, "T 15"

also produces an unusually large number of very minute ascocarps, especially ascocarps with just two asci. Such differences between the Australian fungus and the type culture of *E. glabra* could be an expression of the natural variation within the species. But it is also possible that both organisms have undergone some modifications since they were brought into culture; and particularly it would be surprising indeed if the Dutch fungus had not become changed in some way after nearly a quarter of a century in the laboratory.

A comparison of cultural features in the various *Emericellopsis* entities was reported by Grosklags and Swift (**11**). However, these workers did not consider at all the possible effect of such environal factors as light and temperature on the character of the growth obtained on the various media which they employed. In the present study one striking effect of temperature was observed in the case of *E. humicola*. According to Grosklags and Swift, colonies of this species developing on Czapek-Dox, honey-peptone, and potato-dextrose agars fail to form the salmon pink to golden color which is so characteristic of most other *Emericellopsis* entities. Cain (**3**), who described the species, reported that the organism produces slow-growing, white to grey colonies on malt agar. The writers likewise obtained always only a light grey color in cultures of *E. humicola* grown on the same or similar media at room temperatures. When, however, some Czapek-Dox agar plate cultures two weeks old were moved to a refrigerating incubator at 16° C and to a refrigerator at 6° C, a bright salmon pink color developed throughout the mycelium within two or three days, and the colonies then closely resembled those of several other species. Cultures of *E. humicola* incubated at low temperatures (16°–18° C) throughout their growth period also developed the characteristic pigmentation.

Another interesting environal effect was noted when some plate cultures of various species on Czapek-Dox and modified Czapek-Dox agar (dextrose or cerelose substituted for sucrose) were incubated on the surface of a laboratory table at the same time that corresponding cultures were incubated within cardboard boxes three and one half inches deep, with loosely fitting covers, placed on the same laboratory table. The cultures concerned were all started with hyphal tips; the exposed plates received diffuse light from windows about fifteen feet away; and, in most trials, only a single layer of plates was placed in each container. When the plates were examined at the end of two weeks, it was observed that for several species, notably *E. salmosynnemata*, *E. microspora*, and *E. terricola*, the colonies grown in the open were substantially larger than those which developed within the boxes and in some cases were of

somewhat different topography (FIG. 15). The same phenomenon was later again observed when the two available isolates of *E. glabra* on plates of vitamin-enriched Czapek-Dox agar and vitamin-enriched modified Czapek-Dox agar (see above) were grown side by side in boxes and in the open (FIGS 13, 14). Since there were at least three replicated plates for each species on each medium in each situation, and since the

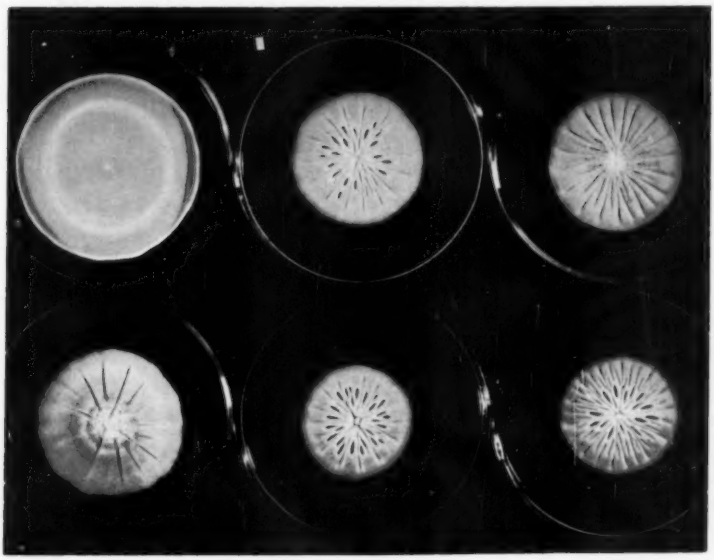


FIG. 15. Pairs of *Emericellopsis* cultures grown in the light and in the dark. The upper member of each pair was incubated in the light at room temperature, 26°–32° C; the lower member of the pair was incubated in the dark at the same temperature. All cultures were started from hyphal tips and colonies were 2 weeks old when photographed. Pair of cultures on the left: *E. salmosynnemata* on modified Czapek-Dox agar (reagent grade dextrose substituted for reagent grade sucrose). Pair of cultures in the middle: *E. microspora* on Czapek-Dox agar. Pair of cultures on the right: *E. microspora* on modified Czapek-Dox agar (reagent grade dextrose substituted for reagent grade sucrose).

colonies on the replicated plates showed very close agreement in size and topography, it is apparent that the organisms concerned were definitely responding to some feature of difference in the environment. Undoubtedly the response was to light; for the only other obvious factor to be considered was a possible small difference in opportunity for gas exchange in the two incubation situations, and it seems unlikely that this could have been responsible for the results obtained.

Much remains to be learned concerning the effects of such environal factors as light, temperature, and aeration on growth, sporulation, antibiotic production, etc. by the various *Emericellopsis* entities. However, it is known that light enhances conidial production in *E. salmosynnemata* and *E. glabra* (**8**), and an elevated temperature (30°–32° C) seems favorable for antibiotic production by some *Emericellopsis* species (**8**, **10**). The effect of environment on the initiation and development of the perfect stage in this genus has been explored to a limited extent by the writers and others at the University of Wisconsin. Our observations have made clear that at least in such species as *E. microspora, E. glabra* and *E. mirabilis,* excellent production of ascocarps can be obtained in darkness as well as in the light and over a wide range of temperatures, provided that the organisms are being cultivated on a suitable substrate.

SUMMARY

A new antibiotic-producing species of *Emericellopsis, E. microspora* Backus and Orpurt, is described. This taxon, which belongs to the small-spored section of the genus, appears to be most closely related to *E. minima* Stolk. The new entity occurs in considerable abundance in the soil of wet prairies in Wisconsin but has not been encountered in any other type of habitat during a 10-year survey of the mycoflora of undisturbed soils of this state.

Emericellopsis terricola var. *glabra* van Beyma is raised to species rank as *E. glabra* (van Beyma) Backus and Orpurt. The substantial differences in morphology and physiology which separate this entity from *E. terricola* van Beyma are recorded. An Australian isolate of *Emericellopsis* which is a strong producer of cephalosporins has been referred to *E. glabra.*

Observations on the effect of certain environal factors on growth and development in various species of *Emericellopsis* are reported.

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STUDIES IN FUNGAL LUMINESCENCE

MARTHA D. BERLINER

(WITH 3 FIGURES)

Luminescent fungi have not held the interest of microbiologists, nor have they fostered the research that has been expended on luminescent bacteria and fireflies. The following are the results of some preliminary studies undertaken to determine the physiology of continuous light production in seven basidiomycetes.

MATERIALS AND METHODS

Dikaryotic cultures of *Armillaria mellea* (Vahl ex Fr.) Kummer, *Armillaria fusipes* Petch, *Mycena galopus* (Pers. ex Fr.) Quél., *M. polygramma* (Bull. ex Fr.) S. F. Gray, *Panus stipticus* (Bull. ex Fr.) Fr., *Clitocybe illudens* (Schw.) Sacc., and *Omphalia flavida* (Cke.) Maubl. & Rangel were maintained in the dark at 22° C on 10% bread crumb agar (1000 ml tap water, 100 g dried commercial bread crumbs without preservatives, 18 g Bacto-agar), a medium found to provide maximum sustained luminescence.

Light intensities were determined from Petri dish cultures covering as nearly as possible the entire agar surface. Because of previously reported periodicity of light emission intensity in three of the species used (**3**), all measurements were made at noon (sun-time) in a light tight chamber using a calibrated Photovolt photomultiplier #520C, with the tube suspended and centered four centimeters above the level of the culture. The response of this very sensitive photomultiplier is in the range of 300–700 mμ, which encompasses the reported emission spectral range of 470–640 mμ for *Armillaria mellea, Mycena polygramma* and *Omphalia flavida* (**7**). The plates were rotated in place 90° and four measurements automatically recorded. Except for the *Armillaria* cultures which spread in a dendritic fashion, the light intensity values for the different areas varied less than 5% and for this study the maximum values in millimicrolumens for five samples from each species are reported.

TABLE I

LIGHT INTENSITY VALUES

Organism	Maximum intensity values*	Time to reach maximum	Period of sustained luminescence
Armillaria fusipes CDA 22484**	730	30 days	10 weeks
Armillaria mellea ATCC 11113	1100	30 days	10 weeks
Mycena galopus	80	14 days	4 weeks
Mycena polygramma	340	18 days	6 weeks
Panus stipticus CDA 35289	855	21 days	10 weeks
Panus stipticus CDA 17798	740	21 days	10 weeks
Panus stipticus CDA 9518	875	21 days	8 weeks
Clitocybe illudens CDA 22424	120	14 days	7 weeks
Omphalia flavida	210	12 days	6 weeks

* An intensity value of 1 is equivalent to 1 millimicrolumen or 5×10^{-8} ft-c.
** Cultures labelled CDA were kindly supplied by Dr. Ruth Macrae.

RESULTS

Light Intensity. Studies of light emission intensities of fungi either in nature or in culture have all been inaccurate and subjective, often depending on the light adaptation of the viewer's eyes, and have consistently been reported to be very low, although no absolute values have been reported to date (**5**). It was first necessary to determine accurately

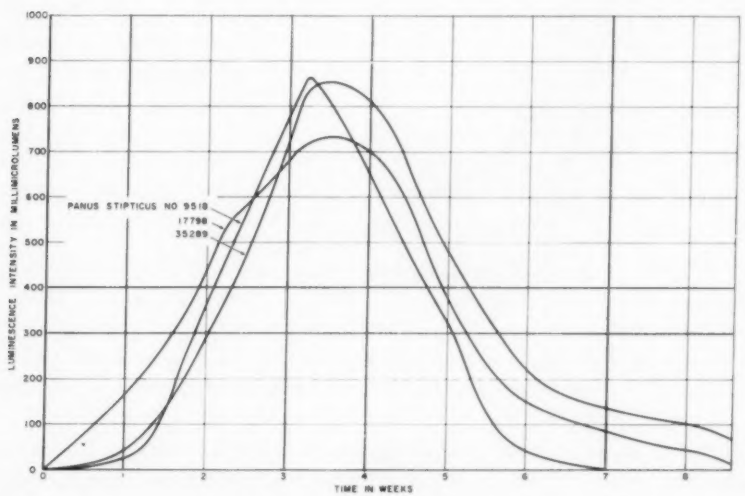

FIG. 1. Luminescence intensity values for a period of nine weeks of three strains of *Panus stipticus* maintained in total darkness. All measurements made daily at noon.

the intensity of the light emission in the seven species before any effects of environmental changes could be assessed.

The data in TABLE I indicate that under favorable cultural conditions the light emission of these fungi is of long duration. The intensities

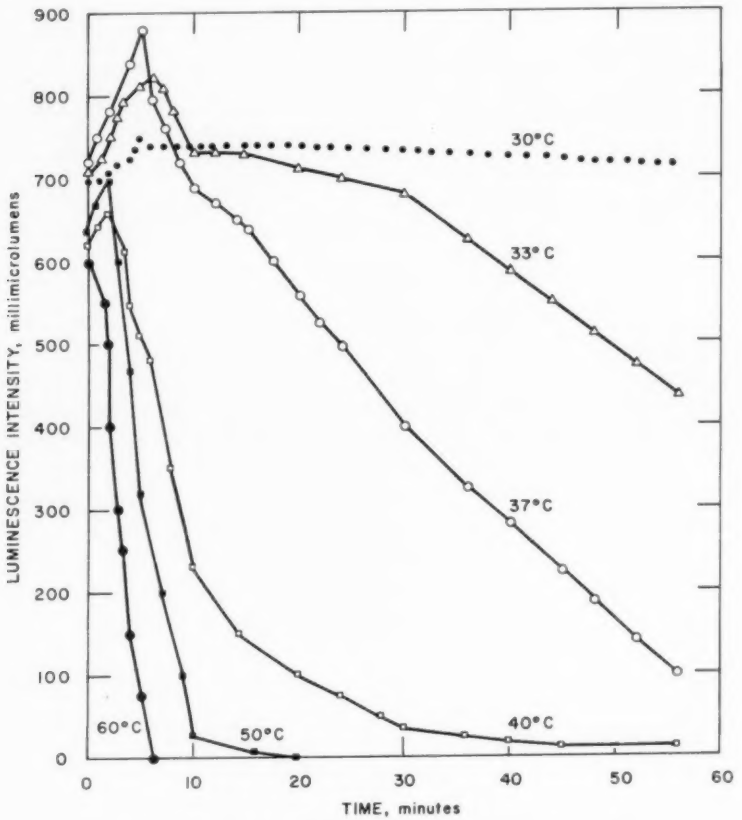

FIG. 2. Effects of increased temperature above optimum for *Panus stipticus* cultures maintained in total darkness.

vary considerably, both between and within species. Three isolates of *Panus stipticus* have close peak intensities and their light intensity curves were very similar (FIG. 1).

Even with long-time dark adaptation differences in intensities of less than 300 millimicrolumens could not be reliably detected visually.

This points to the necessity of accurate photometric measurements of low intensities in studying the effects of different variables.

Although *Armillaria mellea* cultures recorded the brightest light, this species was not used for our further work because of its slow growth and because its dendritic pattern of growth make numerous analyses difficult.

Temperature. Light emission in *Panus stipticus* occurred over a broad range of temperatures. Mature cultures still luminesced at about 5 millimicrolumens after two weeks at – 10° C and fully revived shortly after being removed from the cold. Weak luminescence was also maintained at temperatures above 40° C for two weeks and the light emission returned to normal when the temperature was lowered to 25° C. Above 40° C, the damage quickly became irreversible. The observed optimum range of 18° to 26° C extends the limits previously reported (**4**).

At temperatures higher than optimum (FIG. 2), there was an initial rise in the luminescence intensity followed by a sharp drop. The higher the temperature the sooner the extinction was reached. At 60° C there was no initial rise, and the extinction was irreversible after ten minutes of exposure. These results are similar to those of Airth (**2**) for *A. mellea* at the lower temperatures.

Radiation. Two series of experiments on the effects of X-irradiation on luminescence of *P. stipticus* were conducted. One series was in the range of .3R to 20R and the other 100R to 5000R. All the 14-day-old cultures were irradiated between 11 AM and 1 PM so as to be exposed about midway during the rise in the light intensity curve (**3**). Intensity after irradiation was recorded at 30 second intervals for the first 10 minutes, every 5 minutes for the next hour, and every 30 minutes for the next 3 hours. Thereafter, readings were taken every four hours for five consecutive days—the limit of our observations.

Series I—.3R to 20R. All exposed cultures showed a 2–4-fold increase in luminescence intensity with 2–3 hours, some reaching a peak as early as ten minutes after exposure. This increase was then consistently maintained for at least five days. The time of readjustment to the diurnal cycle varied from 12 to 72 hours. There was no correlation between rapidity of readjustment and intensity of radiation.

After exposure there was a rapid increase in growth for 24–48 hours along the entire perimeter of the colony—about three times that of the controls. This growth was denser and more aerial than normal, and no sectoring was visible. Thereafter, the relative growth rate and pattern were similar to that of the controls.

X-irradiation at these levels of exposure did not affect the blue-green color of the light emission as judged visually.

Series II—100R to 5000R. As with the previous series none of these dosages killed the cultures or extinguished the luminescence.

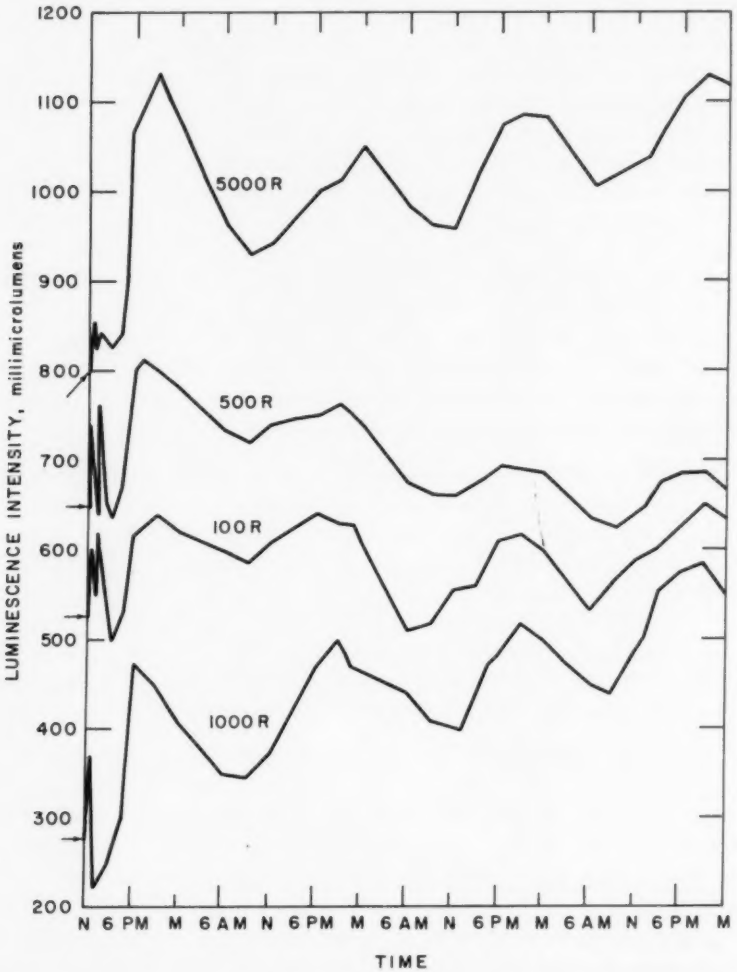

FIG. 3. Luminescence intensity curves for cultures of *Panus stipticus* exposed to various intensities of X-irradiation. Arrows indicate light intensities before exposure. The immediate increases occurred within three minutes of exposure.

There was an immediate (30 seconds-3 minutes) sharp rise in the luminescence intensity followed by one or more drops and rises in the next 3 hours. Within the first 6–8 hours, increases of from 20 to 75% of the original intensity were reached and maintained. This total increase was much greater than expected from the normal diurnal increases (FIG. 3). Again readjustment to the diurnal pattern was fairly rapid as at lower exposures to X-irradiation.

There was no visible difference in the growth rate of exposed and unexposed cultures, nor was there any change in the color of the light.

DISCUSSION

Harvey (**5**) and Airth (**1, 2**) reported the inhibition of fungal luminous systems both *in vivo* and *in vitro* by various chemicals and by ultraviolet light. The effects of X-irradiation on luminescence have only been studied for bacteria by Higginbotham and Bowers (**6**) who noted that all dose levels affected the survival of *Achromobacter fischerii* while the effect of reducing the luminescence intensity did not become apparent until the dose was increased to 5000R. He found no increase in the luminescence as reported here following X-irradiation, or as Airth (**1**) reported for *Armillaria mellea* following exposure to ultraviolet light.

Although the fungal luminescent system is in many ways not comparable to that of bacteria (**1**), it must be remembered that luminescence in the intact cell is dependent on oxygen concentration and the proper functioning of the respiratory system (**6**). Since the survival of *Panus stipticus* did not appear to be affected by X-irradiation doses up to 5000R and the light emission was increased, the following conclusions might be drawn: (a) a radiation-labile component normally partially inhibits light emission or (b) X-irradiation stimulates, either directly or through the formation of by-products, a radiation stable component essential for luminescence. These sustained increases demand further investigations which are now underway in this laboratory.

SUMMARY

Preliminary reports show that luminescent fungi can be useful test organisms for long-time studies in the physiology of light emission, now that simple accurate photometric techniques are available.

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THE EFFECT OF TEMPERATURE ON ASCOGONIAL AND PERITHECIAL DEVELOPMENT IN CERATOCYSTIS FAGACEARUM[1]

F. W. Cobb, Jr., C. L. Fergus, and W. J. Stambaugh

Sexuality in *Ceratocystis fagacearum* (Bretz) Hunt, the oak wilt fungus, is manifested by the formation of ascogonia and conidia which function as the male sex cells, and also by an outbreeding mechanism of compatibility factors. The latter appears to be constant and unaffected by environment since there are no reports of a culture (originating from mycelium, conidia or ascospores) changing compatibility type. Isolates have been reported to have lost the ability to act as a female (**1**, **8**), or to vary quantitatively in their ability to produce perithecia (**3**). Obviously environment can affect the phenomena of ascogonium formation and perithecium development. Stambaugh et al. (**9**) have studied the influence of temperature upon the *in vitro* development of perithecia. However, their study included the influence of temperature only upon perithecial development following conidiation. This process has previously been referred to as spermatization (**5**), but conidiation is a more accurate term (**7**) and will be used herein. A differential effect of temperature upon protoperithecium development and formation of mature perithecia was demonstrated by Hirsch (**6**) with *Neurospora crassa* Shear & Dodge.

This study was carried out to determine the effect of temperature upon ascogonium formation as well as on the events following conidiation, presumably plasmogamy, caryogamy and ascus maturation, of the oak wilt fungus.

MATERIALS AND METHODS

Isolate 53/57 B, obtained from a wilting red oak, *Quercus rubra* L., in the summer of 1957, was used as the ascogonial culture, or female receptive thallus. Isolate 60/58 A was used as the source of conidia for conidiation. It had been isolated from a wilting red oak in the summer of 1958.

[1] Contribution No. 283 from the Department of Botany and Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication July 31, 1961, as Paper No. 2587 in the Journal Series.

Petri dishes containing 40 ml of Barnett's medium (1) were inoculated at the center with single discs of mycelium cut from the advance edges of 12 day-old colonies of the ascogonial isolate grown on Barnett's medium at 20° C. The discs were cut with a sterile cork borer with a cutting diameter of 3 mm.

The inoculated dishes were wrapped in "Saran Wrap" to prevent unequal drying of the agar medium and incubated at various temperatures, TABLE I. The incubators are accurate to within 0.5 degrees. After 14 days incubation, the cultures were conidiated with suspensions of isolate 60/58 A grown for 6 days at 20° on Nutramigen agar (2) slants. The conidial suspensions were adjusted to concentrations so that one drop would yield 500 conidia per cm^2 of colony surface. The colonies attained different sizes when incubated at the various temperatures. The surface area of each colony was determined by tracing the perimeter of the colony on transparent paper and measuring the tracing with a planimeter. By this means the average surface area was accurately determined for the colonies grown at the different temperatures.

The drop of conidial suspension was pipetted onto the receptive colony and then spread over the entire colony by using a sterilized water color brush.

Immediately after conidiating the colonies, all dishes were again wrapped in "Saran Wrap." The colonies grown in each incubator were then divided into four groups at random. Each group received a different temperature treatment as follows: Group 1 was immediately returned to the temperature at which it was incubated before conidiation; Group 2 was placed at 20° for 10 hours and then returned to the original incubation temperature; Group 3 was immediately placed at 24°; and Group 4 was placed at 20° for ten hours before being placed at 24°. These different procedures were followed in order to expose the plates to an optimum temperature for conidial germination (2). One-half of the colonies were placed at 24°, the optimum for perithecial formation (9), so that a comparison could be made of the effect of temperature upon ascogonial formation before conidiation. Each treatment was replicated three times.

Observations were made 3 and 6 days after conidiation by using a dissecting microscope. Data were recorded with reference to the number of fertile perithecia, the number of sterile perithecia (based on absence of a visible mass of extruded ascopores), and the general type of mycelial growth of the colony.

The number of perithecia per colony was converted to the number per square centimeter of colony surface. By using the latter value, the

effect of temperature upon fungus growth could be removed from the evaluation of the effect upon ascogonial and subsequent perithecial development.

TABLE I

EFFECT OF TEMPERATURE UPON ASCOGONIAL AND PERITHECIAL DEVELOPMENT OF CERATOCYSTIS FAGACEARUM

Temp. ° C	Treatment***	Av. colony area (cm²)	P*	S**	P per cm²	S per cm²
10	1	0.5	0	0	0	0
	2	0.4	0	0	0	0
	3	0.5	0	0	0	0
	4	0.5	0	0	0	0
15	1	4.1	11	7	2.7	1.7
	2	4.9	9	5	1.7	1.0
	3	5.5	13	8	2.5	1.5
	4	4.6	8	2	1.8	0.4
20	1	11.3	337	30	29.7	2.7
	2	this treatment is the same as treatment 1			—	—
	3	12.9	252	24	19.3	1.9
	4	9.2	282	9	30.3	1.0
24	1	21.0	485	30	24.2	1.4
	2	13.9	701	54	50.9	1.3
	3	this treatment is the same as treatment 1			—	—
	4	this treatment is the same as treatment 2			—	—
27	1	13.9	26	24	1.9	1.7
	2	11.3	24	45	2.2	4.0
	3	8.8	96	52	10.8	5.9
	4	14.0	129	20	9.2	1.4
30	1	8.2	0	0	0	0
	2	8.7	0	0	0	0
	3	9.4	0	0	0	0
	4	9.7	0	0	0	0

* Average number of fertile perithecia based on 3 replicates.

** Average number of sterile perithecia based on 3 replicates.

*** Treatment 1, colonies immediately returned to the same temperature at which they were incubated before conidiation. Treatment 2, colonies placed at 20° C for 10 hours and then returned to the original incubation temperature. Treatment 3, colonies immediately placed at 24° C following conidiation. Treatment 4, colonies placed at 20° for 10 hours and then placed at 24°.

RESULTS

Ceratocystis fagaccarum exhibited substantial differences in rate of growth at the different temperatures. There were considerable variations in colony size within each temperature treatment. The effect

TABLE II

EXPRESSION OF VARIATION AMONG TREATMENTS*

	15° C				20° C			24° C		27° C			
	1	2	3	4	1	3	4	1	2	1	2	3	4
Variance (single observation)	2.9	0.9	0.1	1.0	17.6	18.1	4.3	32.2	84.6	1.2	1.5	0.4	0.2
Coefficient of variation	0.63	0.56	0.05	0.54	0.14	0.22	0.07	0.23	0.18	0.57	0.56	0.06	0.05

* See footnote of Table I.

of colony size on the number of perithecia produced was reduced by calculating the number of perithecia produced per unit area, TABLE I.

Very little growth occurred at 10° C. Maximum growth occurred at 24° and growth decreased as the temperature was increased above 24°. These results agree with those previously reported by other workers (**4**, **11**). The mycelium at 30° was decidedly aerial as compared to the appressed growth which occurred at the other temperatures.

Temperature affected the numbers of ascogonia produced. No perithecia developed at 10° or 30° even when the cultures were placed at 24° after conidiation. Since 24° is the optimum for perithecial production (**9**), it can be assumed that no ascogonia developed at 10° or 30°.

Colonies grown at 15°, 20° and 27° produced significantly fewer perithecia than the colonies grown at 24°, TABLE III. There was no significant increase in the number of perithecia produced when colonies grown at 15° or at 20° were incubated at 24° after conidiation. The number of ascogonia was the limiting factor because the same numbers

TABLE III

SIGNIFICANCE OF PERTINENT COMPARISONS AS DETERMINED BY THE STANDARD NONPAIRED T TEST

Comparison	t value	Comparison	t value
15° C–20° C	11.553**	20° C_3–27° C_2***	3.411*
15° C_1–15° C_2***	0.862	20° C_4–27° C_4	17.128**
15° C_{1+2}–15° C_{3+4}	0.053	24°C_1–24° C_2	4.268**
15° C_{3+4}–27° C_{3+4}	15.545**	24°C_1–20°C_1	1.350
15° C_{1+2}–27° C_{1+2}	0.231	24°C_1–27°C_3	4.097**
20° C_1–20° C_3	3.034*	24° C_2–27° C_4	7.832**
20° C_3–20° C_4	4.031**	27°C_1–27° C_2	0.029
20° C_4–24° C_2	3.784*	27° C_{1+2}–27° C_{3+4}	14.570**
20° C_3–24° C_1	1.213	27° C_3–27° C_4	3.580*

* Significant at the .05 level.

** Significant at the .01 level; t values with no asterisk are not statistically significant.

*** Subscript refers to treatment.

of perithecia were formed when the colonies were placed at the original temperatures as when they were placed at 24°. There was a significant increase in the number of perithecia when the colonies grown at 27° were transferred to 24° after conidiation. Thus, at 27°, temperature was more limiting for perithecial and ascus maturation than for ascogonial formation.

The results (TABLE I) show that temperature affects conidiation. As already stated, some of the colonies were placed at 20° for 10 hours following conidiation to standardize the effect of temperature upon spore germination. No significant differences were observed among the treatments at 15° or between treatments 1 and 2 at 27°. The difference between treatments 3 and 4 at 27° proved to be significant at the 5% level.

TABLE IV

EFFECT OF TEMPERATURE UPON SIZE OF THE PERITHECIAL NECK OF CERATOCYSTIS FAGACEARUM

Temp. °C	Length of neck (microns)	Width of neck (microns)
15	349* (298–412)**	78* (50–128)**
20	321 (255–391)	78 (64–106)
24	249 (190–305)	88 (50–128)
27	224 (155–305)	87 (57–114)

* Average of ten perithecia.
** Minimum and maximum measured values.

This significant difference was obviously due to the low variability among replicates of both treatments and conceivably would not be significant if the experiment was repeated. The small effects of the 10 hour exposure to 20° in these cases can probably be explained in that the number of ascogonia was so limited by the incubation temperature that the number of conidia which germinated (or fused with the ascogonia) was not a limiting factor.

When the colonies grown at 20° or 24° were placed immediately at 24° after conidiation, the numbers of perithecia produced were significantly reduced as compared to the numbers produced when the colonies were placed at 20° for 10 hours before being placed at 24°. Thus 20° is a more favorable temperature for conidiation than is 24°. The variation among the three replications of each treatment is expressed in TABLE II as variance of a single observation and as coefficient of variation.

During the course of the study it was observed that temperature seemed to have an effect upon the length of the perithecial neck. In order to substantiate this observation, 10 perithecia were removed from each of two colonies grown at each of the four temperatures at which

perithecia matured. The length and width of each perithecial neck were measured, TABLE IV. No attempt was made to measure the entire perithecium. The length was considered to be the distance between the perithecial base to where the ostiolar hyphae flare out from the tip of the neck.

The necks of the perithecia were shorter with increased temperature. The largest difference between successive temperatures occurred between 20° and 24° where a difference of 72 microns was recorded. The widths of the necks were the same at 15° and 20°, and approximately the same at 24° and 27°. A substantial increase in the width occurred with the increase in temperature from 20°–24°. This difference and those in the length of the neck were statistically significant at the 5 per cent level (t test).

DISCUSSION

A differential effect of temperature upon ascogonium formation and fertilization with ascus maturation has been demonstrated in this study. This result agrees with that of Hirsch (**6**) who showed a similar temperature effect for *Neurospora crassa*. It is not unexpected that particular stages of development of ascocarps should be affected differently by temperature since it is a complex process. Wilson (**10**) has described the processes of ascogonium development, perithecial formation and ascus maturation of the oak wilt fungus. He was unable to demonstrate the fusion of a conidium with the trichogyne from the ascogonium. It is not known whether the conidium must put out a germ tube to fuse with the trichogyne; however, in this experiment incubation of conidiated colonies for 10 hours at 20° (groups 2 and 4) should have allowed such germination and subsequent plasmogamy.

An effect of temperature upon conidiation was observed particularly with the colonies grown at 20° and 24°. When such cultures were returned to 24° immediately after conidiation, the numbers of perithecia which were produced were significantly reduced as compared with the numbers produced when the colonies were placed at 20° for 10 hours before being placed at 24°. Thus, although the optimum temperature for ascogonium formation and perithecium maturation is 24°, the optimum for conidiation, presumably plasmogamy, is 20°.

SUMMARY

No ascogonial formation or perithecial development of *Ceratocystis fagacearum* occurred at 10° or 30°, even when the cultures were placed at 24° after conidiation. Colonies grown at 15°, 20°, and 27° produced significantly fewer perithecia than did colonies grown at 24°. These

differences were statistically significant at the 1 and 5 per cent levels (t test). The optimum for conidiation, presumably plasmogamy, is 20°. Temperature affected the length and width of the perithecial necks. The necks were shorter with increased temperature. The width and length differences were statistically significant at the 5 per cent level (t test).

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PHYSIOLOGICAL STUDIES ON CALVATIA SPECIES. I. VITAMIN REQUIREMENTS[1]

M. Sedlmayr, E. S. Beneke, and J. A. Stevens

The genus *Calvatia* has assumed a greater importance in recent years following the discovery by Lucas et al. (1958) that aqueous extracts of some of its species retarded the growth of Crocker Mouse Sarcoma 180 implanted in mice.

A review of the literature revealed no detailed reports on the nutritional-physiological needs of these fungi. Consequently, a portion of the nutritional requirements of these organisms was studied to provide a basis for further investigations of the circumstances under which *Calvatia* species produce chemical substances active against tumors.

The present work is intended to provide some information concerning the physiology of *Calvatia* species. The specific purpose of this investigation was to determine the vitamin requirements of four strains of *Calvatia*.

EXPERIMENTAL PROCEDURE

The four strains of *Calvatia* species studied were chosen because of their different types of mycelial growth patterns and their different responses against Sarcoma 180. The tumor-retarding ability of these fungal extracts was determined by the Sloan-Kettering Institute for Cancer Research, New York City.

The following strains were employed in this study: 1) *Calvatia gigantea* 1019 B, 2) *Calvatia gigantea* 1018 F, 3) *Calvatia gigantea* 766 and 4) *Calvatia fragilis* 1020.

Pyrex Erlenmeyer flasks were used as culture vessels. The glassware was treated with sulfuric acid-dichromate cleaning solution, rinsed with tap water, followed by a distilled water rinse. Each flask received 25 or 40 ml of medium according to the method used. The flasks were stoppered with nonabsorbant cotton and sterilized by autoclaving 15 minutes at 15 pounds pressure.

The completely synthetic basal medium used was Lindeberg's medium: Glucose, 20.0 g; NH_4-tartrate, 5.0 g; KH_2PO_4, 1.0 g;

[1] Contribution number 61-8, Department of Botany & Plant Pathology, Michigan State University, East Lansing, Michigan.

$MgSO_4 \cdot 7H_2O$, 0.5 g; $FeCl_3$ (sol 1/500), 0.5 ml; $ZnSO_4$ (sol 1/500), 0.5 ml; $MnCl_2$ (sol 0.1M), 0.5 ml; $CaCl_2$ (sol 0.1M), 5.0 ml; H_2O distilled 995.0 ml.

The pH was determined electrometrically and adjusted to 5.5 before autoclaving.

A stock solution was made for each vitamin, and the amounts required for each experiment were obtained from this source. This stock solution was kept refrigerated.

Each of the following vitamins was added to separate flasks containing the basal medium in the following quantities: Thiamine hydrochloride 100 μg/liter; pyridoxine hydrochloride 100 μg/liter; biotin crystalline 5 μg/liter; and i-inositol 5000 μg/liter.

Two methods of inoculation and incubation were employed and compared in this investigation.

1. *Floating method* (Lindeberg, **1944**).—The inoculum for the experiment consisted of 3.5 mm discs removed from the periphery of a 14-day old culture grown on "Medium A" agar in Petri dishes. The discs of inoculum were floated on the surface of 25 ml of the liquid medium in 125 ml Erlenmeyer flasks. The inoculated culture vessels were incubated (stationary) at room temperature.

2. *Shake method* (Derrick, **1949**).—In order to obtain reproducible quantitative results among replicate cultures, Kluyver's (**1933**) method was used to prepare a standard inoculum. To produce the inoculum for an experiment, mycelium from stock cultures was fragmented in a Waring Blendor and 1 ml was transferred into 40 ml of "Medium A" solution contained in a 125 ml Erlenmeyer flask. This was then placed for 14 days on a reciprocating shaker having a stroke of 0.5 inches, and producing 100 one-inch excursions per minute.

The mycelial pellets obtained from these 14-day old shake cultures were fragmented for 30 seconds in a sterile Monel metal semi-micro Waring Blendor. The material in the resulting homogenous suspension was washed with sterile distilled water and centrifuged at 4000 rpm for 10 minutes. This procedure was repeated twice to give a total of three washings. The last resuspension was standardized by suspending 1 part mycelial fragments with 30 parts of distilled water. This final suspension was used to prepare a second inoculum (which was to be employed to inoculate the experimental flasks). This was accomplished by putting the first inoculum into Lindeberg's vitamin-free medium and allowing the mycelium to grow for 14 days on the shaker. The mycelium thus produced was then fragmented, washed, centrifuged and resuspended (as described previously), and this constituted the inoculum which was

used for the vitamin studies. It was felt that by employing this technique, carry over of the nutrients could be avoided.

The density of the inoculum was determined with a Klett-Summerson photoelectric colorimeter using the green filter (500–570 mμ). Each flask was inoculated with 1.0 ml of the final blended suspension which gave a reading of 12–15% transmission under the above described condition.

The cultures were incubated at approximately 25° C on a reciprocating shaker (described previously) with no additional aeration other than that caused by the continuous agitation. The cultures were exposed to diffuse daylight with intervening dark periods at night.

Sufficient flasks were inoculated to provide four replicates for each strain harvested after 14 and 28 days on the shaker. The length of incubation necessary for optimum growth for each method was determined previously by running a series of growth curves.

The contents of each flask were filtered on tared Whatmann No. 1 filter paper, using a Buchner funnel. The mycelial pellets were washed with distilled water to remove any excess medium and dried at 96° C for 24 hours.

All quantitative data are based on the average dry weight of mycelium produced in the test medium in quadruplicate flasks. In all the experiments the pH was checked at the conclusion.

Stevens (**1957**) reported the growth of several basidiomycetes in a modified Czapek's-Dox formula which he called "Medium A". It had the following composition: Glucose, 15.0 g; sucrose, 15.0 g; Bacto-peptone, 5.0 g; Bacto-yeast extract, 5.0 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; distilled water, 1000 ml; final pH (not adjusted), 5.5. It was decided to compare this medium, a variation lacking the peptone, and another variation lacking the yeast extract, with growth of the *Calvatia* species in Lindeberg's synthetic medium. These data appear in TABLE III. It will be noted that the flasks containing "Medium A" and its two variations were removed from the shaker at the end of 15 days, whereas the flasks containing Lindeberg's medium plus the added thiamine were allowed to continue growth until the 28th day. This was done owing to the nature of growth (very dense) in the first mentioned media. It was felt that once the flasks were filled with mycelial pellets, it would be unwise to leave them on the shaker for an additional 13 days wherein they would be unable to continue growing owing to the dearth of nutrients. Since the flasks containing vitamins plus Lindeberg's basal medium showed very little growth at the end of 15 days, (and these were part of the larger

TABLE I

THE UTILIZATION OF VITAMINS BY CALVATIA SPECIES GROWN BY SURFACE CULTURE (FLOATING) TECHNIQUES. REPORTED AS THE AVERAGED MYCELIAL DRY WEIGHT OF FOUR REPLICATES

Medium	Strains							
	1018F		1019B		766		1020	
	mg*	pH*	mg	pH	mg	pH	mg	pH
L. & biotin	22	5.3	18	5.3	7	5.5	29	5.3
L. & thiamine	50	4.7	42	4.8	22	5.2	60	4.9
L. & pyridoxine	11	5.2	15	5.5	3	5.5	23	5.4
L. & inositol	10	5.3	18	5.4	2	5.5	20	5.4
L. (control)	11	5.3	11	5.5	3	5.5	16	5.5

* Present in the flask at the end of 28 days incubation at approximately 25° C.
L. = Lindeberg's vitamin-free basal medium.

experiment, reported in TABLE II), we elected not to harvest them at that time, but rather 13 days later.

Following the preliminary experiments, the four isolates of *Calvatia* species were grown in Lindeberg's basal medium (submerged culture method) (Lindeberg, **1944**) to which thiamine had been added to a final concentration ranging from 25 μg/liter to 150 μg/liter. These data appear in TABLE IV.

Since many contradictory reports appear in the literature concerning the relative merits of the floating culture method as opposed to the submerged culture method, it was decided to subject the data we gathered to statistical analysis. This analysis data is reported in TABLE V.

TABLE II

THE UTILIZATION OF VITAMINS BY CALVATIA SPECIES GROWN IN SUBMERGED CULTURE. REPORTED AS THE AVERAGED MYCELIAL DRY WEIGHT OF FOUR REPLICATES

Medium	Strains							
	1018F		1019B		766		1020	
	mg*	pH*	mg	pH	mg	pH	mg	pH
L. & biotin	7	5.0	8	5.2	4	5.5	7	5.5
L. & thiamine	40	4.5	27	4.5	10	5.0	58	5.0
L. & pyridoxine	4	5.0	4	5.5	0	5.5	6	5.5
L. & inositol	2	5.0	3	5.5	0	5.5	4	5.5
L. (control)	6	5.0	4	5.5	2	5.5	3	5.5

* Present in the flask at the end of 28 days incubation at approximately 25° C.
L. = Lindeberg's vitamin-free basal medium.

TABLE III

THE UTILIZATION OF VARIOUS MEDIA BY CALVATIA SPECIES GROWN IN SUBMERGED CULTURE. REPORTED AS THE AVERAGED MYCELIAL DRY WEIGHT OF AT LEAST THREE REPLICATES

Medium	Strains							
	1018F		1019B		766		1020	
	mg*	pH*	mg	pH	mg	pH	mg	pH
"A"	300	5.5	280	6.0	66	5.5	407	5.5
"A" minus peptone	48	4.2	55	4.5	37	5.3	204	6.5
"A" minus yeast extract	250	5.3	140	5.5	47	5.5	492	5.5
Lindeberg's plus thiamine**	40	4.5	27	4.5	10	5.0	58	5.0
Lindeberg's** (control)	6	5.0	4	5.5	2	5.5	3	5.5

* Present in the flask at the end of 15 days incubation at approximately 25° C.
** Data reported at the end of 28 days incubation.

A comparison of the utilization of thiamine and thiamine-pyrophosphate by the four *Calvatia* species is reported in TABLE VI.

DISCUSSION AND RESULTS

The experiments with the different vitamins showed that all four strains of *Calvatia* studied were totally deficient for thiamine (TABLES I, II). Upon the addition of crystalline thiamine (100 μg/liter of medium) a good growth of mycelium was produced. The addition of biotin, pyridoxine or inositol singly to the basal Lindeberg's medium did not add measurably to the growth and when it did, only a small

TABLE IV

THE EFFECT DIFFERENT CONCENTRATIONS OF THIAMINE HAD UPON THE GROWTH OF CALVATIA SPECIES GROWN IN SUBMERGED CULTURE. REPORTED AS THE AVERAGED MYCELIAL DRY WEIGHT OF FOUR REPLICATES

Concentrations*	Strains							
	1018F		1019B		766		1020	
	mg**	pH**	mg	pH	mg	pH	mg	pH
25 μg/liter*	45	4.5	23	4.6	10	5.2	67	5.0
50 μg/liter	48	4.5	28	4.5	11	5.2	70	4.9
100 μg/liter	48	4.5	27	4.5	11	5.2	69	4.9
150 μg/liter	49	4.5	30	4.5	11	5.2	74	4.9

* Added to Lindeberg's synthetic basal medium.
** Present in the flask at the end of 30 days incubation at approximately 25° C.

TABLE V

A STATISTICAL COMPARISON OF THE YIELDS OBTAINED USING SURFACE CULTURE AND SUBMERGED CULTURE METHODS. FIGURES INDICATE DRY WEIGHT OF THE MYCELIUM EXPRESSED IN MG

	Strains															
	1018F				1019B				766				1020			
	A		B		A		B		A		B		A		B	
	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$	$\bar{X}$	$S_{\bar{x}}$
Biotin	7	1.2	22	4.3	8	0.9	18	6.2	4	0.3	7	1.3	7	1.3	29	7.5
Thiamine	40	4.5	50	6.0	27	0.7	42	5.2	10	0.9	22	5.4	58	1.0	60	6.1
Biotin & thiamine	37	3.7	49	7.8	25	1.2	36	2.8	9	0.7	19	2.5	44	5.4	56	9.3
Pyridoxine	4	0.6	11	1.8	4	1.1	15	3.3	0	0	3	1.1	6	0.5	23	4.3
Inositol	2	1.1	10	2.9	3	1.1	18	3.4	0	0	2	0.6	4	0.6	20	2.8
Control	6	0.9	11	1.9	4	0.6	11	2.4	2	0.4	3	0.6	3	0.6	16	1.6

Legend:
A = submerged culture method.
B = surface culture method.
$\bar{X}$ = arithmetic mean (average dry weight of replicates).
$S_{\bar{x}}$ = standard deviation of the mean, calculated by the following formula:

$$S_{\bar{x}} = \frac{\sqrt{\Sigma(x - \bar{x})^2}}{n(n-1)}.$$

amount of sparse mycelium developed. Consequently, it appears that *Calvatia* species are heterotrophic with respect to thiamine. Luxurious growth depends on an external supply of this growth factor.

According to Melin and Nyman (**1940**) all of the species of basidiomycetes they studied were heterotrophic with respect to thiamine but

TABLE VI

THE UTILIZATION OF THIAMINE AND THIAMINE–PYROPHOSPHATE BY CALVATIA SPECIES. REPORTED AS THE AVERAGED MYCELIAL DRY WEIGHT OF FOUR REPLICATES

	Strains							
	1018F		1019B		766		1020	
	mg	pH	mg	pH	mg	pH	mg	pH
Thiamine: 150 μg/liter								
14 days	13.5	5.1	18.5	5.2	5.3	5.3	10.5	5.1
28 days	37.1	4.5	26.9	4.7	11.9	5.1	55.0	4.7
TPP: 150 μg/liter								
14 days	12.7	5.2	20.0	5.0	6.5	5.3	11.7	5.0
28 days	39.3	4.5	28.1	4.6	11.7	5.0	52.3	4.7

autotrophic or self-sufficient with respect to biotin, when grown in nutrient solution. Fries (**1955**) found several degrees of thiamine deficiency in *Coprinus* species.

Judging from the data which appear in TABLE III, there is an indication that in addition to the thiamine requirement, *Calvatia* species have a partial deficiency for an unidentified growth factor(s) present in Bacto-peptone and Bacto-yeast extract. In "Medium A" the four strains of *Calvatia* had 7–10 times more mycelium on a dry weight basis than in Lindeberg's medium plus thiamine. Alternative explanations may be that 1) the four vitamins studied are present in Bacto-peptone and Bacto-yeast extract in ratios which are ideal for the growth of these organisms, or 2) different concentrations than were used in this report are required.

After comparing the chemical components of the two media, one concludes that the slightly greater amount of sucrose in "Medium A" could not be responsible for the differences in yields. The presence of an unknown growth factor in Bacto-peptone and Bacto-yeast extract seems to be evident because of the favourable effect on growth following the addition of 5 grams of each of these two products per liter to a medium containing an adequate amount of available carbon, nitrogen and trace elements. The hypothetical substance is apparently water soluble and thermostable since it is resistant to autoclaving. It may be a single compound but more likely several.

The presence of unidentified growth factors in natural media is not an unknown phenomenon, as evidenced by various reports. Yusef (**1953**) found that the addition of small amounts of malt extract to a basal medium containing vitamins and casein hydrolysate markedly increased the growth of different *Polyporus* species. He suggested that the presence of unidentified growth stimulating factors in malt extract ("malt factor") appeared to play a role in the nutrition of this fungus. Melin and Das (**1954**) observed that roots of pine and other plants exert a strong growth promoting effect on tree-mycorrhizal basidiomycetes. They concluded that the roots produce one or more metabolites designed as "factor M," which are essential to the growth of these fungi. This growth factor could not be replaced by either the vitamins of the B complex group, the amino acids in casein hydrolysate, or the components of hydrolysed yeast nucleic acid. Melin (**1959**) reported that a mixture of nucleic acid components, however, seemed to lower the growth-promoting effect of the "M factor."

Upon studying the data reported in TABLE III, it is noticeable that with one exception (strain 1020), *Calvatia* strains gave the best growth

when Bacto-peptone and Bacto-yeast extract were both present in "Medium A." However, in all four cases the yield was much lower when Bacto-yeast extract was the sole additive with Bacto-peptone lacking. The Bacto-yeast extract data reported above may be due to the pH changes of media during incubation (in the absence of the buffering effect of Bacto-peptone) or it may be an actual inhibitory effect by the Bacto-yeast extract which can be overcome by the addition of Bacto-peptone. Judging from the results obtained with strain 1020, the latter supposition seems to be more likely because, in spite of the buffering effect of Bacto-peptone, the organism had better mycelial growth in the absence of Bacto-yeast extract. Fries (**1950**) mentioned the superiority of yeast to thiamine alone for the growth of some basidiomycetes, but he emphasized the fact that yeast extract contains inhibitory compounds as well as unidentified beneficial substances.

Increasing the thiamine concentration from 25 to 150 μg/liter of medium did not significantly alter the amount of mycelium produced by the four strains of *Calvatia* species, TABLE IV.

From the data, TABLE V, it is evident that in spite of the differences in the amounts of the mycelial yields given by the floating and shaker techniques, both methods demonstrated that the *Calvatia* strains studied required thiamine. It seems that mycelial growth in the presence of different growth factors was proportionally similar in both methods. It is noticeable that greater amounts of mycelium are produced using the floating method than in the shaker method. According to the literature, this is assumed to be due to the "carry-over" of nutrients by the inoculum, Derrick (**1949**), Foster (**1949**) and Wiken et al. (**1951**), which is unavoidable when using mycelial discs. There may be present in the discs a certain amount of nutrient materials and growth factors which influence the growth of the colony. If this were not so, then the amount of fungal growth produced by the shaking method would not be so much lower, since the dry weight of both forms of inocula was roughly the same. However, another explanation may be possible. It must be kept in mind that the submerged culture method employed fragmented mycelium as the inoculum. As such, even though the numbers of cells present in both inocula may be approximately equal, the possibility exists that fragmentation may have disrupted the cell walls of a large number of the cells, thus rendering them nonviable.

Another disadvantage of the floating method is also evident from the data, TABLE V. The replicates of the floating method show a greater deviation from the mean value than is observed in the case of the shaker method. The great variability produced by the floating method is ex-

pressed in the consistently higher standard deviation ($S_{\bar{x}}$) values, TABLE V.

The results, TABLE VI, indicate that there are no significant differences in the utilization of thiamine and thiamine-pyrophosphate by the four strains of *Calvatia* tested. Leonian and Lilly (**1940**) compared the action of thiamine and thiamine-pyrophosphate in several thiamine deficient fungi. They found no difference in the maximum weights of mycelium formed in the presence of equivalent quantities of these two growth factors. Therefore, they concluded that cocarboxylase can replace thiamine, possibly entering the cell without dephosphorylation occurring. Jansen (**1954**) reported cocarboxylase to be about 30 per cent more active than equimolecular quantities of thiamine for most microorganisms. Lilly and Barnett (**1951**) suggested that pyruvate accumulates in the culture medium of some thiamine-deficient fungi in the absence of the required amount of thiamine. The pyruvate accumulation was accompanied by a decrease of pH in the medium. This was not observed in the case of *Calvatia* species. In the absence of thiamine, the pH of the medium did not decrease which may suggest that pyruvic acid, instead of accumulating in the medium, could be further metabolized by any one of several possible pathways, except the Krebs cycle.

SUMMARY

Three strains of *Calvatia gigantea,* 1019 B, 1018 F and 766, and one strain of *Calvatia fragilis* 1020 were studied in this nutritional investigation.

All four strains of *Calvatia* grew very well on a modified Czapek-Dox medium, and adequately on Lindeberg's medium to which thiamine had been added.

The utilization of the nutrients was determined by the growth response of the organisms and measured on the basis of the averaged mycelial dry weight of three or four replicates after two to four weeks of incubation at approximately 25° C.

Each of the strains investigated was totally heterotrophic with respect to thiamine, and autotrophic with respect to biotin, pyridoxine and inositol. The action of thiamine seemed not to be quantitative within certain limits because an increase in the concentration caused no significant increases in the growth. A partial deficiency was established for an unidentified growth factor(s), present in Bacto-peptone and Bacto-yeast extract.

No significant differences in the mycelial growth of the strains of

Calvatia were obtained when equivalent amounts of thiamine and thiamine-pyrophosphate were compared.

The submerged culture method (using fragmented hyphae) proved to be superior to the surface culture method, as statistical comparison of the averaged mycelial yields produced by each method showed the surface culture method to have a much higher standard deviation value.

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Britonia, quarterly; beginning in 1937, official publication of the American Society for Plant Taxonomists. $7.50 a year.

Economic Botany, quarterly; review and research articles dealing with plants useful to man; established 1947; beginning 1959, the official journal for The Society for Economic Botany. $5.00 a year.

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The Mycoflora of North America. By Robert Hagelstein. 1944. $7.50.

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